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The geographical distribution of this race was found to be in Madhya Pradesh, Rajasthan, Bombay State, Mysore State and Andhra Pradesh—thus, so far restricted to Peninsular India.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

Race 17 of brown rust of wheat: A new record for India—L. M. Joshi, Gopal Swarup, K. R. Sreekantiah and R. S. Vasudeva. A new race, resembling race 17 of *Puccinia triticina* Eriks. of International key has been isolated from various collections of rust, from the crop year 1956–57 from India. In the very first year of its appearance in the country, the race was recorded from the states of Uttar Pradesh, Bihar, Madhya Pradesh, Bombay, Madras and Andhra. Its frequency was 12.7 per cent during the year.

Reactions of one of the isolations found in India are compared with race 17 of the International key in the following table:

Locality and Original host	Stock Collection or Insolation	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat
Wellington Wheat var.?	Brevit (1–2)	4	0;–2	0;–2	0;–1	0;–2	0;–1	4	0;–1
——	Race 17	4	0	0	0	0	0	4	0

Race 17 has been picked up from most of the states of the country during subsequent years.

Division of Mycology and Plant Pathology
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Race 162 of brown rust of wheat: A new record for India.—D. P. Misra, V. C. Lele and R. S. Vasudeva. During the analysis of brown rust samples collected from crop year 1955-56, a sample from Gwalior (Madhya Pradesh) on wheat variety N.P. 720, yielded race 162. Reactions of the eight differential varieties to the isolate from India and to the race 162 of the International key are set out in the following table for comparision:

Locality and Original host.	Stock collection or Isolation	Malakof	Carina	Brevit	Webster	Lotos	Mediterranean	Hussar	Democrat
Gwalior (M.P.) N.P. 720	Mal. (2)	0;–2	4	4	3	4	4	X	4
——	Race162	I	3	4	4	4	4	X	4

Race 162 is different from race 77 for its resistant type of reaction on Malakof. Reactions on other seven differential varieties are more or less identical with these two races. All other races previously known in India i.e. 10, 11, 20, 26, 63, 70, 106, 107, & 108 are uniform for the resistant type of reactions produced on Mediterranean and Democrat which varieties are susceptible to races 77 and 162.

Race 162 has shown increasing prevalence in subsequent crop-years.

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THE MYXOMYCETES OF INDIA-XIII

K. S. Thind and H. S. Sehgal

(Accepted for publication January 10, 1960)

"The Myxomycetes of the Mussoorie Hills". have been published in the form of 12 papers (listed under references) describing 78 known species, 5 new species and one new form. The present contribution deals with ten species collected from the Darjeeling Hills (2,000–8,500. a s. l.) in the Eastern Himalayas, five of which are new records for India.

The classification of Martin, 1949, has been followed throughout this study, although monographs of Lister and Lister, 1925, and Macbride and Martin, 1934, were freely consulted.

The number of the species shall now designate the serial numbers of the myxomycetous flora of India.

Type collections have been deposited in the Herbarium of the Panjab University and Herbarium Crypt. Ind. Orient., New Delhi.

The authors are deeply indebted to Prof. P. N. Mehra, Head of the Panjab University Botany Department, for encouragement and providing facilities. They are also thankful to Mr. B. Khanna for making illustrations of fruit bodies.

85. *Badhamia capsulifera* (Bull.) Berk.

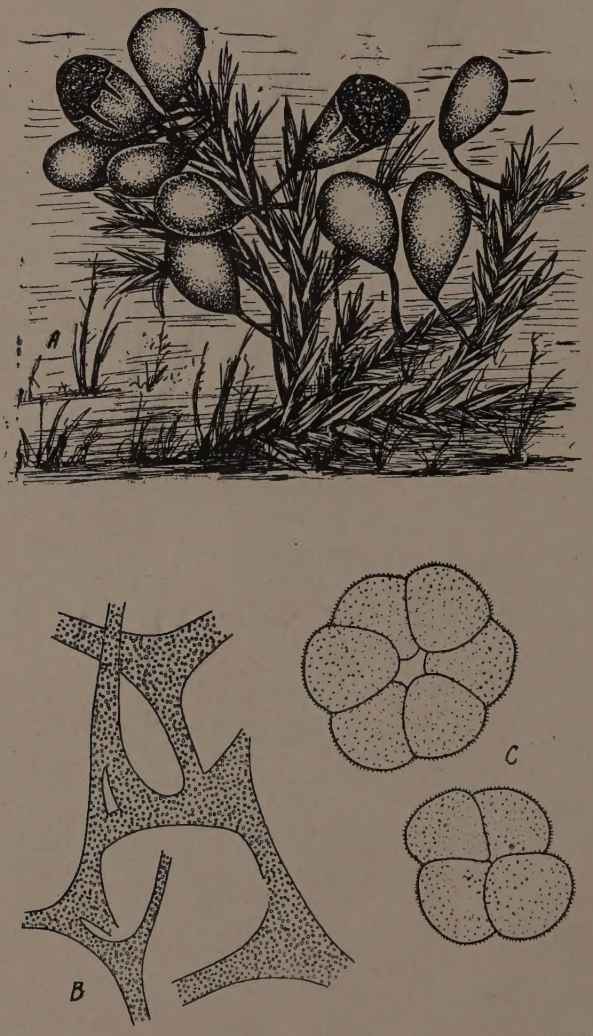
Fructifications sporangiate, total height 1.5–1.9 mm. ; sporangia 0.4–0.8 mm. in diameter, densely and widely clustered, stipitate, bent to prostrate, ovoid or pyriform, blue-grey, or iridescent violet; stipe 0.6–1.6 mm. long, very weak, often repent to prostrate, membranous, strand-like, straw-coloured, gradually narrowed above, longitudinally rugose; hypothallus indistinct; peridium single, thin, membranous, smooth or rugulose, iridescent, hyaline when empty; dehiscence irregular.

Columella absent.

Capillitium abundant, composed of a delicate, open network of slender calcareous tubules, slightly expanded at the angles, white, persistent.

Spores 11.2–14 μ in diameter, black in mass, dark brown under the microscope, adhering in persistent clusters of 5–15, broadly ovate, strongly

verrucose on the exposed surface, minutely to inconspicuously verrucose on the covered surface in the interior of the balls. Text-Fig. 1, A-C.



Text...Fig. 1. *Badhamia capsulifera* (Bull.) Berk.,
A. Sporangial clusters, with slender weak stipes, x 20. B. Capillitium, x 400. C. Persistent clusters of verrucose spores with warts most prominent on the exposed surfaces, x 1150.

Collected on alive mosses and bark of a stump, Tiger Hill, Darjeeling, August 1, 1958, 333. New record in India.

This very beautiful Darjeeling collection undoubtedly belongs to *Badhamia capsulifera* (Bull.) Berk. but it combines the characters of *B. utricularis* (Bull.) Berk. in possessing similar iridescent bluish-grey peridium, large clusters of sporangia, and strand-like, straw coloured, prostrate stipes.

86. *Craterium minutum* (Leers) Fries

Fructifications sporangiate, total height 1–1.3 mm.; sporangia 0.4–0.7 mm. long and 0.2–0.4 mm. in diameter, gregarious to densely gregarious, stipitate, erect or slightly bent, longitudinally wrinkled, brownish yellow to yellowish brown to reddish brown or deep chocolate, in some cases upper portion being darker than the lower one, goblet shaped or cyathiform, depressed at the top, margin of the cup thickened and everted; lid depressed throughout, fragile, paler coloured than the peridium, greyish white to brownish, margin raised up to 0.15 mm. above the lid; stipe 0.5–0.9 mm., stout, erect or slightly bent, brownish yellow, dark brown near the base, concolorous with the sporangium above, longitudinally rugose, uniformly wide, noncalcareous; hypothallus small, distinct, dark brown, rotate, radially rugose; peridium composed of two thick layers (except at the margin above the lid), closely applied to each other: outer layer cartilaginous, darker coloured, everted at the rim; inner layer lighter coloured, ends at the depressed lid (thus being absent at the rim), brownish yellow, translucent, thick; dehiscence by a distinct lid; lid depressed throughout, thin, delicate, greyish white, calcareous, of different structure and texture from the peridial wall.

Columella absent.

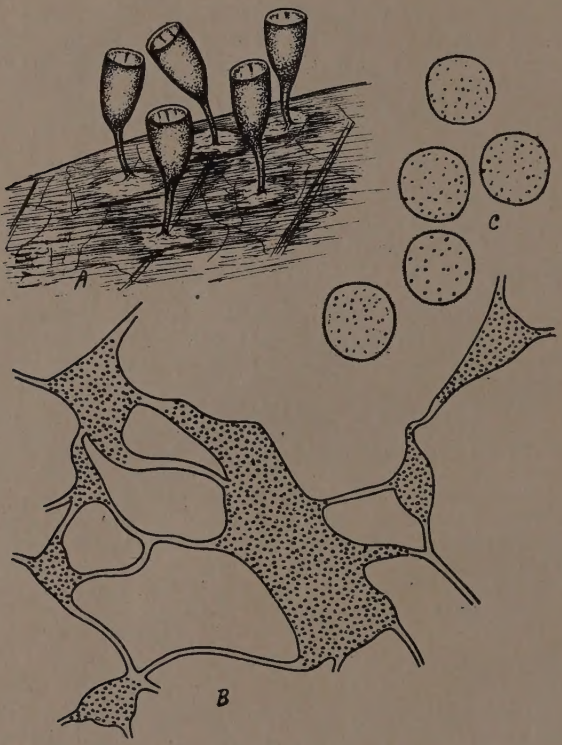
Capillitium physaroid, well developed, attached to the peridial wall all round, composed of nodes and internodes; nodes white, calcareous, irregular in shape and size, often aggregated together in the centre, interconnected by slender, hyaline, noncalcareous, thread-like internodes.

Spores 9–10.5 μ in diameter, black in mass, violaceous brown under the microscope, globose, very minutely verrucose, thick walled. Text-Fig. 2, A-C.

Collected on dead leaves, Lebong forest, Darjeeling, July 30, 1957, 334.

This fungus evidently belongs to *Craterium minutum* (Leers) Fries and is characterized by the cyathiform, brownish sporangia, white nodes and thick walled very minutely verrucose spores (9–10.5 μ in diameter). The distinct lid is depressed throughout below the mouth of the sporan-

gium in all the Darjeeling material examined. The peridium is apparently single but its wall is distinctly composed of two layers both of which are quite thick in the Darjeeling collection.



Text...Fig. 2. *Craterium minutum* (Leers) Fries.
A. Sporangia, x 20. B. Capillitium, x 400.
C. Minutely verrucose spores, x 1150.

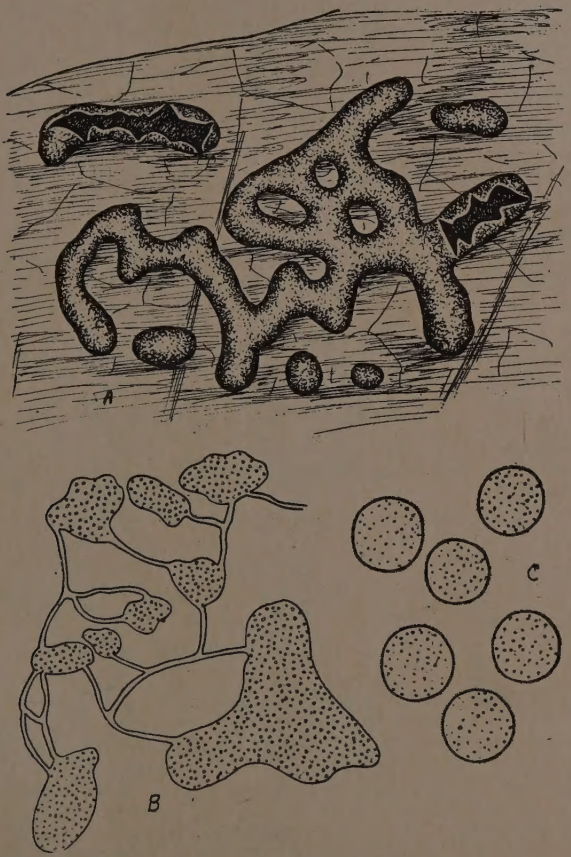
87. *Physarum superbum* Hagelst.

Fructifications predominantly plasmodiocarpous with a few sporangiate types, gregaricus to clustered in small groups or scattered, orange coloured due to orange calcareous deposit which is predominantly developed on the top and is almost lacking on the sides, or orange yellow; plasmodiocarps 0.2–0.5 mm. wide, 1–7 mm. long, short or long, straight or bent, arcuate, flexuous, or forming small irregular nets, usually terete, sometimes laterally compressed; sporangia 0.2–0.6 mm. in diameter, small, sessile, globose to subglobose; hypothallus absent; peridium single, thick, rugulose, pelliculose, yellowish green, covered at the top with small clusters of minute orange calcareous granules; dehiscence irregular, lower part persistent.

Columella absent.

Capillitium abundant, persistent, composed of a network of nodes and internodes; nodes abundant, yellow to pale orange to almost pallid coloured or almost whitish, appearing only white under the binocular, orange tinge becoming visible under the microscope, spherical and angular, unequal, calcareous, 10–45 μ in diameter, interconnected by slender, hyaline, noncalcareous internodes, often expanded at points of branching.

Spores 8–10.8 μ in diameter, black in mass, pale violaceous brown to violet to dark violet under the microscope, globose to subglobose, minutely and profusely verrucose, warts sometimes tend to be arranged in clusters as well. Text-Fig. 3, A-C.



Text...Fig. 3. *Physarum superbum* Hagelst.,
A. Predominantly plasmodiocarpous frutcifications, x 20.
B. Capillitium, x 400. C. Spores, x 1150.

Collected on dead leaves, Badamtam, Darjeeling, August 5, 1958, **335**. New record in India, on dead leaves of *Agave* sp., Aduncа Bridge, Mussoorie, September 8, 1956, **335A**.

These beautiful collections evidently belong to *Physarum superbum* Hagelst. Its nodes are whitish to yellow to pallid coloured.

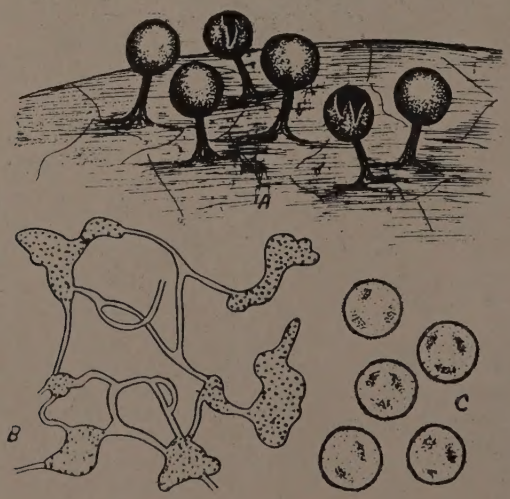
88. *Physarum leucopus* Link.

Fructifications sporangiate, total height 0.6–0.9 mm.; sporangia 0.4–0.5 mm. in diameter, gregarious, stipitate, erect, globose, slightly umbilicate below, greyish white; stipe 0.3–0.5 mm. long, erect, snow white, later becoming cream-coloured, slightly narrowed upward, longitudinally rugose, calcareous, brittle; hypothallus represented by calcareous, white to cream coloured ridges or threads; peridium single, thin, membranous, calcareous, lime in small frosty flakes; dehiscence irregular, lower peridium persistent.

Columella absent.

Capillitium abundant, lax, composed of a network of nodes and internodes; nodes abundant, white, calcareous, large, angular, irregular in shape and size, interconnected by slender, hyaline noncalcareous, long internodes.

Spores 7–9 μ in diameter, black in mass, violaceous brown under the microscope, minutely but distinctly verrucose, marked also by clusters of darker and bigger warts. Text-Fig. 4, A-C.



Text—Fig. 4. *Physarum leucopus* Link, A. Sporangia, x 20. B. Capillitium, x 400. C. Verrucose spores also marked by clusters of darker and larger warts, x 1150.

Collected on dead leaves, Lebong forest, Darjeeling, July 20, 1958. **336**. On dead leaves, Birch Hill, Darjeeling, July 13, 1958, **337**. New record in India.

These Darjeeling collections undoubtedly belong to *Physarum leucopus* Link and are marked by calcareous stipes, lax capillitium with large angular nodes and long internodes, and minutely verrucose spores, 7–9 μ in diameter. Small clusters of darker warts observed in these collections have not been recorded for the species.

89. *Diderma chondrioderma* (de Bary & Rost.) G. Lister

Fructifications sporangiate to plasmodiocarpous, the latter predominating, scattered to gregarious, white, smooth, surface very much like a polished porcelain, transversally depressed; plasmodiocarps 0.6–1·7 mm. in diameter, expanded and lobed, flexuous, sometimes annulate; sporangia 0.6–0.8 mm. in diameter, sessile, globose-depressed; hypothallus absent; peridium single, thin, membranous, covered over by thick, calcareous, shell-like crust formed by rounded lime granules; dehiscence irregular.

Columella well developed, flesh-coloured, roughened, discoid in sporangial forms and columnar in plasmodiocarps.

Capillitium abundant, coarse, brownish purple, composed of sparingly branched stout threads, often with membranous expansions at the joints, membranous expansion often perforated.

Spores 10.5–13.3 μ in diameter, mostly 12–13 μ, black in mass, brownish purple under the microscope, aggregated together in loose spore balls (up to over 50 spores in a ball), globose, profusely and minutely verrucose. Text-Fig. 5, A-C.

Collected on alive mosses on the bark of a tree, Senchel forest, Darjeeling, September 3, 1958, **338**. New record in India.

This interesting Darjeeling collection closely resembles *Diderma chondrioderma* (de Bary & Rost.) G. Lister in all respects except that its spores are mostly aggregated in loose spore balls (spherical to irregular). This feature appears partly due to immature fructifications which were dried out before maturity.

90. *Diderma rugosum* (Rex) Macbr.

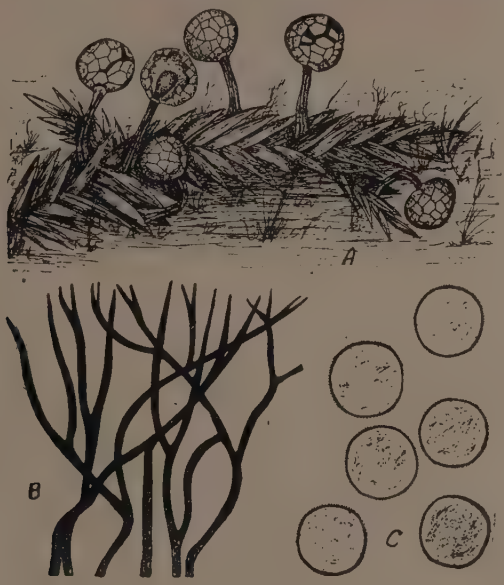
Fructifications sporangiate, total height 0.7–1.2 mm; sporangia 0.4–0.6 mm. in diameter, scattered to somewhat gregarious, stipitate, erect, globose to subglobose, white, brownish at the base; stipe 0.4–0.8 mm. long, well developed, erect, black, gradually narrowed upward, longitudinally rugose or furrowed; hypothallus inconspicuous; peridium single, thick, well charged with lime and hence white, prominently reticulately ridged; dehiscence irregular, the ridges marking the lines of dehiscence into polyhedral fragments.

Text—Fig. 5. *Didermа chondrioderma* (de Bary & Rost.) G. Lister A. Fructifications, x 20. B. Coarse, stout capillitial threads often with perforated expansions at the joints, x 400. C. Spores, x 1150.

Columella clavate, massive, wrinkled, calcareous, white, reaching nearly the middle of sporangium.

Capillitium abundant, violet, hyaline at the apices, composed of forking and anastomosing threads, radiating from the base of sporangium and the columella and joined with the peridium above, noncalcareous, attenuated at the apices.

Spores 8.4–9.8 μ in diameter, black in mass, violaceous brown under the microscope, globose, minutely verrucose. Text-Fig., 6, A-C.



Text—Fig. 6. *Diderma rugosum* (Rex) Macbr., A. Sporangia marked by reticulately ridged peridium, x 20. B. Capillitium, x 400. C. Spores, x 1150.

Collected on alive moss, growing on the wall of Himalayan Glory Hotel, Gandhi Road, Darjeeling, August 26, 1958, 339.

This interesting Darjeeling collection undoubtedly belongs to *Diderma rugosum* (Rex) Macbr. The species is marked by the characteristically reticulately wrinkled peridium, black stipe and white massive columella.

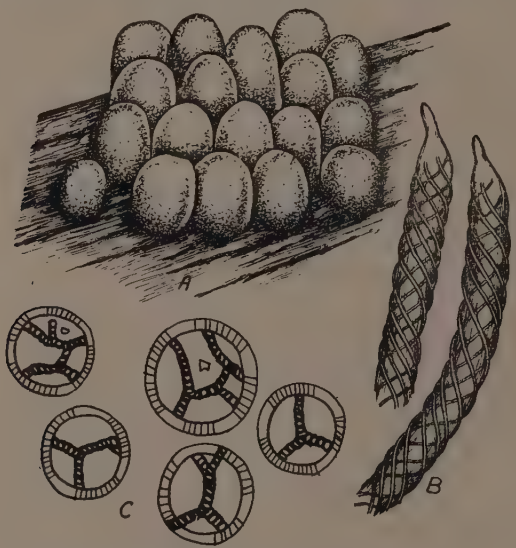
91. *Trichia affinis* de Bary.

Fructifications sporangiate; sporangia 0.4–0.6 mm. in height and 0.3–0.5 mm. wide, closely crowded together into small to large clusters (clusters up to 1.2 cms.) globose to obovoid to ovoid, sessile, pulvinate,

golden yellow; hypothallus membranous, common to a cluster; peridium thin, membranous, translucent, pale yellow, marked by minutely poroid or punctate surface; dehiscence irregular, peridium rupturing at the top, its lower part remaining persistent.

Capillitium abundant, composed of free, elaters; elaters yellow, long, 4–6 μ wide, marked by 4–5 spiral bands, spirals smooth, apices pointed and conical (or short apiculate).

Spores 13.5–15.7 μ in diameter, dull yellow in mass, bright yellow under the microscope, large, globose, marked by coarse, usually complete reticulations with 3–5 meshes to the hemisphere, the reticulate bands broad and prominently pitted and up to 1 μ high, spore surface also marked by distinct lines as seen in sectional view. Text-Fig. 7, A-C.



Text—Fig. 7. *Trichia affinis* de Bary,
A. Sporangial cluster, x 20. B. Parts of elaters, x 1150. C. Spores marked by coarse, reticulate and pitted bands, x 1150.

Collected on bark and wood of a stump and on alive mosses growing on it. Senchel, Darjeeling, September, 1, 1957, **340.**

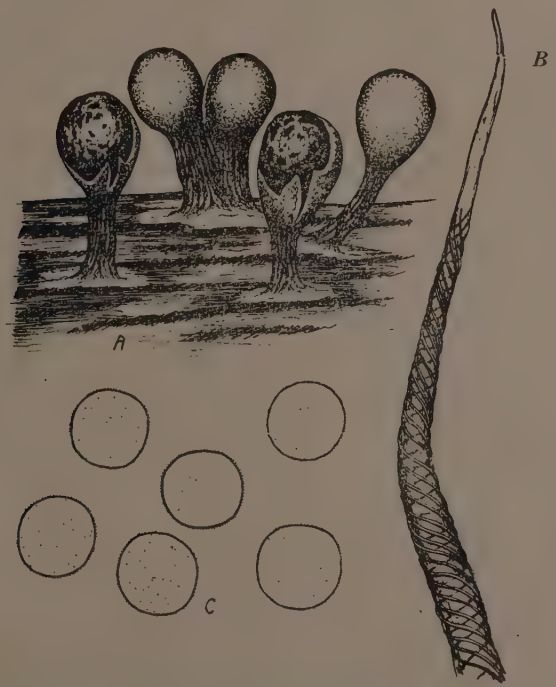
This very interesting collection undoubtedly belongs to *Trichia affiinis* de Bary and is characterized by golden yellow pulvinate sporangia arranged in clusters, free, yellow, long elaters marked by smooth spirals, and large spores marked by a coarse, and usually complete reticulation, the reticulate bands broad, coarse and distinctly pitted.

92. *Trichia botrytis* (J. F. Gmel.) Pers.

Fructifications sporangiate, total height 1–1.5 mm.; sporangia 0.6–0.8 mm. in diameter, gregarious to densely gregarious, sometimes clustered by the fusion of stalks, stipitate, erect, obovate, greyish brown; stipe 0.3–0.9 mm. long, erect, stout, reddish brown, opaque, cylindrical, filled up with amorphous material, longitudinally ridged; hypothallus brown to reddish brown, small; peridium double; outer peridium granular, often separating before dehiscence and forming areolae separated by the lighter inner wall; inner peridium membranous, yellowish brown; dehiscence irregular.

Capillitium abundant, composed of free elaters; elaters long, yellowish brown in mass, bright pale yellow individually, convoluted, 4.9–6.3 μ wide at the centre, drawing into long, acuminate apices, marked by 4–6 spiral bands, spiral bands absent at the extremeties, spirals smooth.

Spores 10–12 μ in diameter, yellowish brown in mass, light yellowish brown under the microscope, globose, profusely and distinctly verrucose. Text-Fig. 8, A-C.



Text—Fig. 8. *Trichia botrytis* (J. F. Gmel.) Pers.
A. Sporangia with stout, longitudinally ridged stipes, x 20.
B. Part of an elater, x 1150. C. Spores, x 1150.

Collected on the bark of a tree, Senchel forest, Darjeeling, September, 3, 1958, **341**. On alive mosses growing on the bark of a tree, Tiger Hill, Darjeeling, August 20, 1958, **342**. New record in India.

Both these Darjeeling collections belong to *Trichia botrytis* (J. F. Gmel.) Pers., no. 342 possessing wider elaters and less prominently marked spores. Spores of no. 341 appear to be more prominently marked by warts than reported for the species.

93. *Trichia floriformis* (Schw.) G. Lister

Fructifications sporangiate, total height 1.5–3.5 mm.; sporangia 0.5-0.9 mm. in diameter, densely gregarious, mostly separate, sometimes united into clusters by union of the stalks, stipitate, erect, turbinate or pyriform, purplish red to nearly black; stipe 0.8–2.5 mm. long, erect to bent, deep red, longitudinally ridged, the ridges running over the base of the sporangia as well, translucent when mounted on a slide; hypothallus deep red to almost black, small; peridium single, cartilaginous, thick, black; dehiscence by irregular rupturing of the peridium at the top, its lower part splitting into petaloid lobes remaining attached to the top of the stipe.

Capillitium abundant, composed of free elaters; elaters brick red in mass, fading to brownish red, individually red, highly convoluted and entangled, gradually tapering at both ends to acuminate apices, unbranched, 4–6 μ wide in the middle, marked by 4–5 spiral bands, spirals smooth.

Spores 9.8–12 μ in diameter, brick red in mass, reddish brown under the microscope, globose, profusely verrucose, warts minute but distinct, uniguttulate, gutta large and filling three-fourth of the spore cavity. Text-Fig. 9, A-C.

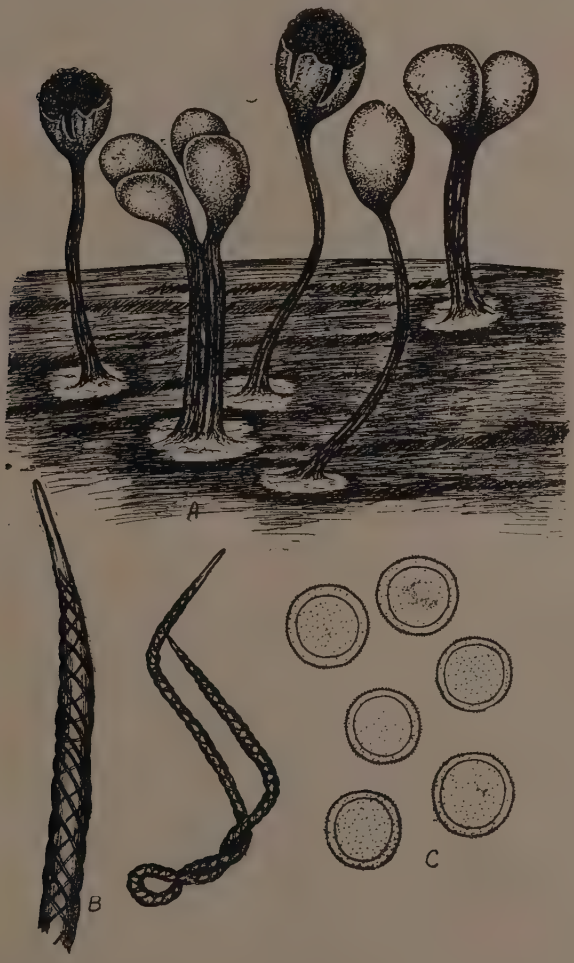
Collected on decaying wood as well as on alive mosses growing on it. Bhanjang forest, Darjeeling, August 28, 1958, **343**.

The inner peridium which is reported for this species has not been observed in the Darjeeling collection. It may be very fusely attached with the outer peridium. This species is easily differentiated from *Trichia botrytis* (J. F. Gmel.) Pers. in possessing darker peridium, brighter and translucent stalk, and its petaloid dehiscence.

94. *Cornuvia serpula* (Wigand) Rost.

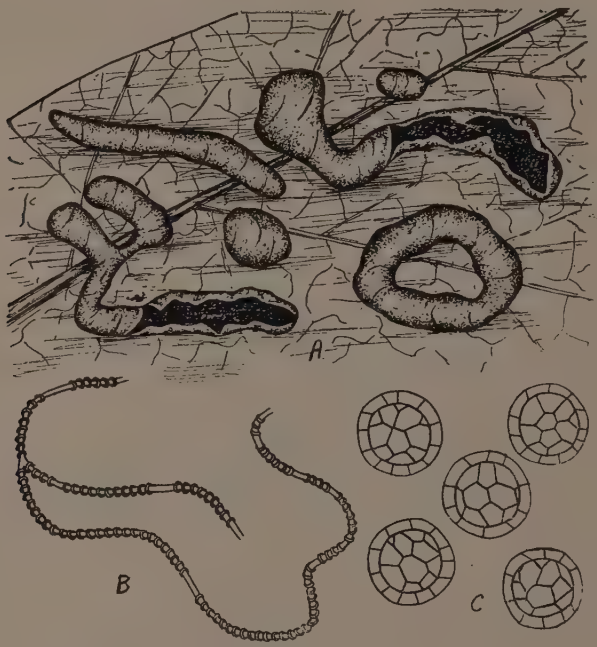
Fructifications mostly plasmodiocarpous, sometimes sporangiate, with all the intergradations, gregarious to scattered, golden yellow; plasmodiocarps 0.2–0.6 mm. in diameter, mostly short, straight to bent to arcuate or flexuous, sometimes annular, sometimes long and branched but not forming a net; sporangia 0.3–0.5 mm. in diameter, globose to subglobose, sessile or pulvinate; hypothallus absent; peridium single, thin, membranous, yellow; dehiscence irregular, peridium rupturing at the top while the lower part of the peridium remaining persistent.

Capillitium abundant, flaccid, composed of yellow, long, sparingly branched, highly convoluted threads so as to form a network; threads up to 3 μ in diameter, prominently and profusely marked by rings; rings large, complete, situated mostly at very short intervals and clustered (usually 3 μ apart), 6–7.5 μ in diameter, concolorous with the threads.



Text-Fig. 9. *Trichia floriformis* (Schw.) G. Lister.
A. Long stipitate sporangia, x 20. B. Part of an elater (x 1150) and a complete elater, convoluted on itself, x 400. C. Uniguttate, verrucose spores, x 1150.

Spores 13–16 μ in diameter including the border, 9–12 μ in diameter excluding the border, border 1.5–2 μ wide, deep yellow in mass, brownish yellow under the microscope, globose, conspicuously reticulated, 8–10 meshes to a hemisphere. Text-Fig. 10, A-C.



Text—Fig. 10. *Cornuvia serpula* (Wigand) Rost.

A. Plasmodioscarps, x 20. B. Branched capillitial thread marked by prominent complete rings, x 400. C. Reticulated spores, x 1150.

Collected on dead leaves and dead twigs, Lebong forest, Darjeeling, July 31, 1957, 344.

This Darjeeling collection undoubtedly belongs to *Cornuvia serpula* (Wignad) Rost. except that its capillitium is not freely branched as reported for the species.

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COMPARATIVE STUDIES ON SCLEROTIUM ROLFSII SACC. AND OZONIUM TEXANUM NEAL & WESTER VAR. PARASITICUM THIRUMALACHAR

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Introduction. The seriousness of the 'Sclerotium rot' disease caused by *Sclerotium rolfsii* Sacc. has been universally acclaimed and that the 'Ozonium rot' caused by *Ozonium texanum* Neal & Wester var. *parasiticum* Thirum. has also been reported to be serious wherever it occurs. The two fungi appear to be similar apparently and produce the same type of pathological reactions on the hosts. It has been stated by Neal and Wester (1934) and Mishra (1952) that *Ozonium* produces fan shaped mycelial growth around the collar region of the affected plant but it seems that this character which distinguished it from *S. rolfsii* is not a constant character to rely upon to separate the two fungi. Higgins (1922, 1927) and Endo (1940) made valuable contributions to the knowledge of physiology and parasitism of *S. rolfsii*, but little is known about the physiological and pathological behaviour of *O. texanum* var. *parasiticum*. The taxonomic studies of the two fungi show some differences like the cell structure and the sclerotial size and colour, in culture, but the similarities in the pathogenetic reactions on various hosts suggest their close relationship. The present investigations were, therefore, undertaken to study the similarities and the variations existing between these two taxonomically distinct fungi, regarding their morphology, physiology and pathogenicity for a better understanding of their systematic position, as also the control of serious plant diseases caused by them.

Material and Methods: The following isolates of *S. rolfsii* and *O. texanum* var. *parasiticum* were included in the present studies:

1. S_1 — Isolated from Pigeon-pea (*Cajanus cajan*) collected from Botanical Farm, I.A.R.I., New Delhi in 1951.
2. S_2 — Isolated from *Piper betle* by S. R. Sen from Bengal in 1932.
3. S_3 — Isolated from *Orobanche* sp. by R. P. Malik in 1944.
4. S_4 — Isolated from Sugarcane by B. B. Mundkur in 1934.
5. S_5 — Isolated from Lucerne by R. S. Mathur in 1941.
6. S_6 — Isolated from *Piper betle* by M. S. Phulbari in 1940 from Sylhet, Assam.
7. S_7 — Isolate received from M. Curzi in 1932. Information regarding host and locality not available.
8. S_8 — Isolate received from Whetzel in 1932. Information regarding host and locality not available.
9. S_9 — Isolated from Guar (*Cyamopsis tetragonoloba*) collected from Botanical Farm, I.A.R.I., New Delhi in 1952.

10. 0 — *Ozonium texanum* var. *parasiticum*, received from the Director, C.P.R.I., Patna, isolated from potatoes in 1951.

Isolates S_2 to S_8 were obtained from the Indian Type Culture Collection, Division of Mycology and Plant Pathology, I.A.R.I., New Delhi. (Accession Nos. 99, 77, 25, 266, 243, 23 and 24 respectively).

Morphological Studies: The morphology of the two fungi presented some very constant differences. The average cell size of the *Ozonium* isolate was 60 x 5.3 μ while that of *S. rolfsii* isolates was 184 x 5.8 μ. The different isolates of *S. rolfsii* were found to maintain their individual characteristics within the restricted range of their cells and sclerotial size and colour. The cell size in isolates S_3 and S_7 measured 103 x 5.3 μ while isolates S_2 and S_8 measured 210 x 5. 8 μ. The mycelial growth in the two fungi was almost similar, except that the *Ozonium* isolate developed fan shaped, stranded growth and that the clamp connections were not observed. In *S. rolfsii* isolates the growth rate was variable, clamp connections, though rare, were observed only in the broader cells; the mycelium was usually coarse, with large cells, flocculose and at times formed strands also.

The sclerotia were formed within 6 to 10 days in both the fungi on various culture media at 30 – 34°C. They are small, round bodies of whitish colour when young but turn to buff brown, clove brown or chocolate brown in case of *S. rolfsii* isolates and yellowish brown, cadmium brown or buff brown in *Ozonium* isolate at maturity. The sclerotial size, colour and production varied with the isolate. The *Ozonium* sclerotia were larger in size, 1.5 to 3.2 mm., and hence a smaller number was formed in a petri plate culture. The production of sclerotia of *S. rolfsii* isolates varied from 108 to 384 in the same unit area and their size varied from 0.9–1.4 mm. to 1.4–2.5 mm. The sclerotia of *S. rolfsii* isolates S_1 and S_2 formed short, massive stalk or cushions, while the *Ozonium* sclerotia sometimes developed pits or depressions on their surface. A colourless drop of liquid was frequently observed to be exuded by the sclerotia of the two fungi, which, however, did not seem to be a constant character. At any rate, in all cases the size, colour and number of sclerotia of the two fungi remained markedly distinct from each other as also for the individual isolates of *S. rolfsii*.

Cultural Studies: In the preliminary cultural studies all the ten isolates of two fungi were grown on oat meal agar, potato dextrose agar, Brown's synthetic medium and Richard's solution agar. Oat meal agar medium was found to be most suitable for growth of all these isolates, while the two synthetic media showed very poor growth and sclerotial formation was also very much reduced. Isolates S_2, S_5 and S_7 showed comparatively lower rate of growth than others. Isolates S_1 and S_4 were comparable to the growth rate of *Ozonium* isolate. The ratio of sclerotial production was found to be proportional to the growth rate of these isolates. A selection of three isolates was made out of the nine isolates of *S. rolfsii* on the basis of their growth character, sclerotial formation and 'aversion' studies (discussed later), for detailed study and com-

parison with *Ozonium* isolate. Isolates S_1, S_2 and S_4 were thus selected for all further studies.

(a) *Effect of Media:* Three *S. rolfsii* isolates and one *Ozonium* isolate were grown on sixteen media* consisting of seven natural media, five semi-synthetic media and four synthetic media. It was observed that all the natural media were good for their growth as well as sclerotial formation, while synthetic media were just the contrary. However, semi-synthetic media proved slightly better than the synthetic media. Takahashi (1927), Higgins (1927) and Endo (1940) had also observed that synthetic media did not provide a suitable substrate for the growth of *S. roflsii* isolates, and all the physiological studies were conducted by them mostly on natural media. Further, it was seen that increase in the quantity of medium in the petri dish culture increased sclerotial production appreciably, but did not increase the vegetative mycelial growth.

(b) *Temperature Relationship:* The four selected isolates were grown on potato-extract agar, corn steep-liquor medium and Richard's medium at various temperatures and it was found that the two fungi were almost alike in their growth response to temperature. Both could grow on a wide temperature range of 16 to 37 °C; with the optimum at 30 to 34 °C on all the three media. The formation of sclerotia was maximum at 30 °C for *Ozonium* isolate while for S_1 and S_4 isolates of *S. rolfsii* the corresponding temperature was 25 °C and for S_2 isolate 30 °C. When the vigorously growing cultures at the optimum temperature are exposed temporarily to either higher or lower temperatures of 40 °C or 10 °C for two days and then transferred back to the optimum conditions, it was observed that the sclerotial production was increased by 2 to 4 times as compared to the controls, in all the four isolates. Further, the exposure of the growing mycelium to the sunlight for 4 or 8 hours daily was found to be inhibitory to the mycelial development but the sclerotial formation was 1.5 to 3 times greater in all the four isolates.

(c) *Effect of Hydrogen-ion concentration:* For pH relationship studies all the four isolates were grown on Richard's medium and potato dextrose agar adjusted at various pH ranging from 2.5 to 9.2. It was observed that the isolates of both the fungi had a wide range of toleration of the pH variations, especially towards acidic side. The *Ozonium* isolate could grow at pH 3.5 to 8.6 with an optimum for vegetative growth at pH 6.0 to 6.2, and maximum number of sclerotia were formed at pH 7.2. In the case of *S. rolfsii* isolates the pH range for growth was found to vary slightly with different isolates. Isolates S_4 showed maximum toleration of pH range, from 2.5 to 9.2, while isolate S_1 could grow only between pH 3.2 and 8.0. However, for all the three isolates of *S. rolfsii* pH 6.6 was found to be optimum for the vegetative growth while the maximum number of sclerotia were produced at pH 6.6 by isolates S_1 and S_4 and at pH 7.2 by isolate S_2. As regards the size of the sclerotia at

* Potato extract agar, Carrot agar, (Goto, 1933), Onion agar, (Goto, 1933), Oat-agar, Maize agar (Curzi, 1931), Tomato tissue agar, (Curzi 1931), Tomato juice agar, Onion asparagin proteose-peptone agar (Mundkur, 1934), Potato dextrose agar, Corn steep-liquor agar, Malt extract agar, Yeast extract agar, Asparagin glucose agar, Richard's synthetic agar, Czapeks' synthetic agar, Brown's synthetic agar.

pH 7.2 the sclerotia of *Ozonium* isolate were found to be reduced in size and measured 0.9 to 1.9 mm. and approached very near to the sclerotial size of *S. rolfsii* isolates. No change in sclerotial size was, however, observed for the isolates of *S. rolfsii* at any pH range. The data regarding vegetative growth are presented in Fig. 1.

In the case of liquid medium, it was found that the pH of the solution changed rapidly to acidic side in the first few days of the fungi growth, after which it gradually became almost neutral. The maximum acidity reached in the case of *S. rolfsii* isolates was pH 3.6 and the *Ozonium* isolate pH 4.0 within 7 to 8 days. Thereafter a gradual reversal was followed and after two months' time the reaction of the culture medium changed to pH 6.3 in the case of *S. rolfsii* isolate and to pH 6.1 for *Ozonium* isolate. Similar observations have been reported by Higgins (1927) also. The experimental results are set forth in Fig. 2.

(d) *Nutritional Studies:* In order to study the basic requirement of different forms of nutrients for healthy growth of the two fungi, basic Richards' medium was used along with its variations in which one of its constituents were eliminated. The two fungi showed more luxurient growth on the medium devoid of magnesium sulphate than the complete medium. In the case of *Ozonium* isolate, the growth was poor on the media devoid of phosphate, nitrate or iron chloride, and the growth on the medium devoid of carbohydrate was only slightly less than the growth obtained on the complete medium. The behaviour of *S. rolfsii* isolates was generally comparable with *Ozonium* isolate, but the absence of carbohydrate from the medium affected the growth to a slightly greater extent. The data are presented in Fig. 3.

(i) *Effect of different carbohydrate sources:* The unusual response of both the fungi to the carbohydrate in their growth led to further investigations of their effect on their vegetative growth. Lilly and Barnett's (1951) basic synthetic medium was used and seven different carbohydrates viz., arabinose, glucose, fructose, lactose, maltose, sucrose and starch, representing different groups, were incorporated at the rate of two per cent in the basic synthetic medium in place of sucrose. It was observed that the growth of all the four isolates was, in general, less in the media containing carbohydrate than that devoid of carbohydrate source and that in the case of lactose there was a marked reduction in growth. However, for *Ozonium* isolate a slight increase in the growth was observed when arabinose, starch or fructose was used. The sclerotial formation was generally correlated with the growth rate of the isolates of the two fungi, except that no sclerotia were found by the *Ozonium* isolate in the medium containing arabinose as a carbohydrate source. The data are presented in Table I.

(ii) *Effect of different nitrogen sources:* Five different nitrogen sources were incorporated in the Lilly and Barnett's basic synthetic medium for studying the response of the isolates of two fungi. It was found that *S. rolfsii* isolates showed slightly better response to all the nitrogen sources tested than the *Ozonium* isolate. Asparagin seemed to be the best nitrogen source for their growth while glycine, ammonium

nitrate and potassium nitrite were found to be fairly suitable. The presence of glycine in the medium inhibited the sclerotial production in isolates S_2 and S_4 of *S. rolfsii*. However, no sclerotia were found by any of the isolates in the absence of nitrogen source.

TABLE I. Influence of different Carbohydrate sources on the growth rate and sclerotial formation of the isolates of *S. rolfsii* and *O. taxanum* var. *parasiticum*.

Culture Medium	Isolate 0		Isolate S_1		Isolate S_2		Isolate S_4	
	Diameter of colony (mm)	No. of sclerotia	Diameter of colony (mm)	No. of sclerotia	Diameter of colony (mm)	No. of sclerotia	Diameter of colony (mm)	No. of sclerotia
Basic with arabinose	61	Nil	56	98	25	20	62	99
Basic with glucose	45	28	40	32	22	24	61	30
Basic with fructose	62	71	56	98	27	73	68	84
Basic with sucrose	53	54	57	104	28	70	68	112
Basic with lactose	21	16	20	20	11	16	16	21
Basic with maltose	48	18	46	53	24	50	50	62
Basic with starch	61	68	62	112	26	78	58	103
Basic without sugar	58	70	60	118	28	78	60	109

(iii) *Effect of C/N Ratio:* To study the effect of C/N ratio various combinations of different concentrations of asparagin (0.1 to 0.4%) and sucrose (0.5 to 2.5 %) in the medium were tried and it was observed that a combination of 0.2 per cent asparagin and 0.5 per cent sucrose gave the optimum growth in all the four isolates.

(e) *Viability of Sclerotia.* It was seen that the sclerotia of both fungi, kept under laboratory conditions gave out profuse mycelial growth when put for germination even after nine months storage, while in nature their viability depended on the depth in the soil at which they were lying. At the end of six months the sclerotia of *Ozonium* isolate buried at the depth of 10–12 cm. showed only 75 per cent viability and at the end of nine months, the viability was only 45 per cent. At the depth of 12 to 15 cm, these sclerotia after weathering for nine months showed 20 per cent germination. In the case of *S. rolfsii* isolates, the viability

after nine months burial at a depth of 12 to 15 cm, was about 40 per cent. Further, it was seen that the sclerotia of *S. rolfsii* were not killed even at 85 °C when heated for ten minutes, but they were killed at 75 °C when heated for one hour, in the dry conditions. In the wet condition, however, the sclerotia were killed at a much lower temperature-in 10 minutes at 55 °C and in one hour at 40 °C. In the case of sclerotia of *Ozonium* isolate, the exposure at 80 °C for ten minutes, at 75 °C for 60 minutes were sufficient to kill them in dry conditions, whereas in the wet conditions they were killed at 55 °C in ten minutes or at 40 °C in about an hour. Further, it was found that the sclerotia of the two fungi lost their viability if kept immersed in water for 72 hours. The data are presented in Tables II & III.

TABLE - II. Effect of temperature on the viability of sclerotia of *S. rolfsii* and *O. texanum* var. *parasiticum*.

a. Under dry conditions: No. of sclerotia tested = 15.

Isolate	Time of treatment minutes	No. of sclerotia germinated after exposure at						
		50 °C	60 °C	65 °C	70 °C	75 °C	80 °C	85 °C
0	10	15	15	14	12	9	4	0
	25	15	15	14	10	7	0	0
	45	15	13	11	9	6	0	0
	60	15	13	10	0	0	0	0
S_1	10	15	15	15	13	9	5	1
	25	15	14	13	10	7	3	0
	45	15	14	12	9	6	0	0
	60	14	15	12	9	5	0	0

b. Under wet conditions: No. of sclerotia tested = 10

Isolate	Time of treatment (minutes)	No. of sclerotia germinated after exposure at					
		30 °C	35 °C	40 °C	45 °C	50 °C	55 °C
0	10	8	6	4	3	1	0
	25	6	7	5	2	0	0
	40	7	4	2	0	0	0
	60	5	3	0	0	0	0
S_1	10	9	8	7	7	3	0
	25	8	9	6	3	0	0
	40	9	7	6	2	0	0
	60	7	4	0	0	0	0

TABLE III. Effect of flooding of soil on the infection of Guar seedlings by *S. rolfsii* and *O. texanum* var. *parasiticum*.

Number of plants (1 month old) used = 12

Time flooded (hours)	Plants infected with	
	Isolate 0	Isolate S_1
12	11	12
24	12	12
36	8	10
48	5	8
72	1	3

The sclerotia of the two fungi were exposed to 30 different inorganic or organic chemicals (1–2 per cent strength), for 12 or 24 hours and then tested for their viability. It was found that chemicals like acetic, carbolic, and picric acids; iron sulphate; mercuric chloride, carbon tetrachloride; carbon bi-sulphide; chloropicrin; ethanol; nitrobenzene and benzol were fatal to the sclerotia, while, citric, hydrochloric and sulphuric acids and formalin afforded some growth of the sclerotia of *S. rolfsii* only. At lower concentrations (0.5% and below), however, oxalic, lactic, succinic, tartaric acids and glycine, ammonium oxalate, ammonium sulphate, ammonium chloride, sodium chloride, sucrose, glycerine, ethanol and chlroform showed some germination of the sclerotia of both the fungi. The action of ammonia vapours on sclerotia was studied in detail and it was observed that 50 ppm. (parts per million) solution of ammonia for 24 hours killed the sclerotia of both the fungi. With the increase in concentration to 100 ppm. it took only six hours exposure for *S. rolfsii* and only one hour exposure for *Ozonium* sclerotia to get killed.

TOXIC PRINCIPLE OF THE FUNGI: In the preliminary hydrogen-ion concentration studies it was observed that the pH of the liquid substrate became acidic soon after the growth of the two fungi, suggesting thereby the formation of metabolic products of acidic nature, which, on analysis was found to be oxalic acid. The cultural filtrate, 25 days old growth when put on the young Guar (*Cyamopsis tetragonoloba*) seedlings at the collar region, was able to produce symptoms similar to those produced by the action of the fungus or even with a solution of pure oxalic acid. The presence of free oxalic acid cystals was also observed in the diseased host tissues. Further, volumetric estimations were made of the culture filterates and it was found that the age of the filtrate presented an index of acid production, which was found to be maximum in 7 to 8 days old cultures. This fact is also supported by the evidence obtained during the study of change of pH reaction of the filterate as already discussed.

PATHOLOGICAL STUDIES: In the host range studies twenty plant species belonging to different families, were found to be easily infected by the two fungi. Within 3 to 5 days of inoculations the plants started

wilting. No differentiation could be observed in the pathogenicity by the different isolates of *S. rolfsii* or *Ozonium texanum* var. *parasiticum*.

It was also observed that the host plants were not affected at low temperatures and that the optimum infection was obtained at 25 to 32°C. High humidity was found to be another pre-requisite for successful infection since no infection could be obtained under dry condition. The infection by the two fungi also depended on the age of the plants. One month old plants showed maximum infection and that with the increase in age the percentage infection decreased.

The position of the inoculum, in the form of dormant sclerotia in the soil and the soil reactions were the other two vital factors which predispose the plants to infection. As already indicated, the depth of soil at which the sclerotia lie gave an indication of its survival and viability, since sclerotia lying at a depth of 12–15 cm. were of little consequence in bringing about infection. The pH of the soil seemed to exert strong influence on the germination of sclerotia and infection: at pH 4.8 to 7.3 the infection obtained was about 100 per cent whereas at pH 8.6 the percentage infection was 40 to 50 for both the fungi and at pH 9.4 the infection was reduced to 10 per cent in the case of *S. rolfsii* and to zero per cent for *Ozonium* sp.

Discussion: The two fungi, *Sclerotium rolfsii* and *Ozonium texanum* var. *parasiticum*, showed distinct morphological differences from each other, while on the other hand their uniform behaviour in different physiological and pathological reactions, bring them very close to each other. Even the different isolates of *S. rolfsii* maintained their individuality under cultural conditions but exhibited no sign of any physiologic specialization when tried on different hosts. Nakata (1925, 1927) and Curzi (1931, 1931a) stated that there were several species confused under the common name of *S. rolfsii*. Taking into consideration the wide host range and distribution of *S. rolfsii*, in all parts of world and their variability, it does not seem justifiable to group all the isolates under one species.

The fact that a rich mycelial and sclerotial crop was obtained mostly on natural media and not on any of the several synthetic media tried, indicated the absence or paucity of certain essential constituents necessary for their growth. The temperature requirements for the optimum growth and sclerotial formation, were different for both the fungi. Maximum sclerotial formation was obtained at 25 to 30°C while 34°C was the optimum temperature for their vegetative mycelial growth. Sudden rise or fall of the temperature as also exposure to sun brought about increased sclerotial production. Both the fungi were found to grow over a wide pH range, 3.2 to 9.2, but the growth was distinctly better on acidic side. These factors explain, to some extent, the world wide distribution of these fungi.

The nutritional studies indicated that the carbohydrates were of little use in the growth of these fungi which is rather unusual. The presence of nitrates, phosphates and iron compounds seemed to be some

of the essential constituents for their normal growth. Presence of lactose retarded the growth rate and ammonium tartrate was not suitable as nitrogen source. Asparagin at the rate of 0.2 per cent and sucrose 0.5 per cent maintained a balance of carbon - nitrogen sources.

The principal function of the sclerotia i.e. to tide over the unfavourable conditions, could be attributed to their great tolerance of fairly high temperatures (upto 85 °C). However, when exposed to ammonia vapours, they were readily killed. These findings are similiar to those reported by Leach & Davey (1935) and indicate the possibility of use of ammonium fertilizers as one of the measures for control.

Conclusive results had been obtained to demonstrate that the toxicity caused to the host by these fungi was due to the metabolism of oxalates. The thermostability of oxalates, their presence in the diseased tissues and in the culture filterate of these fungi further go to support the view that oxalates produced by these fungi are the active principle of their parasitic activity.

From the pathological studies evidence has been obtained regarding the wide host range of the two fungi. Though Epps *et al* (1951) and Allison (1953) demonstrated that the pathogenicity of the different isolates of *S. rolfsii* could be distinguished to some extent by their pathological reactions, yet no physiological forms could be distinguished.

Moderate soil temperature, high soil moisture, high humidity and young age of the plant have been found to be the predisposing factors that contribute to the incidence and intensity of the disease caused by these fungi.

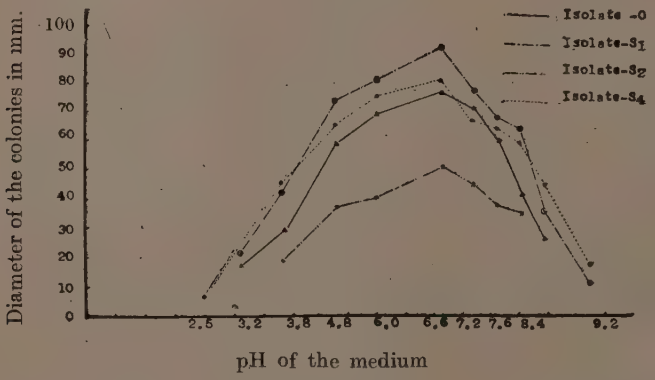

Fig. 1. Effect of Hydrogen-ion concentrations on the growth rate of the isolates of *S. rolfsii* and *O. texanum* var. *parasiticum*.

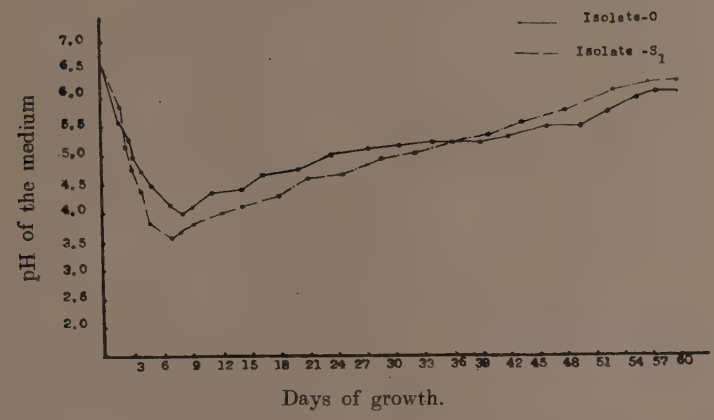

Fig. 2. Course of change of reaction of potato decoction after the daily growth of *S. rolfsii* and *O. texanum* var. *parasiticum*.

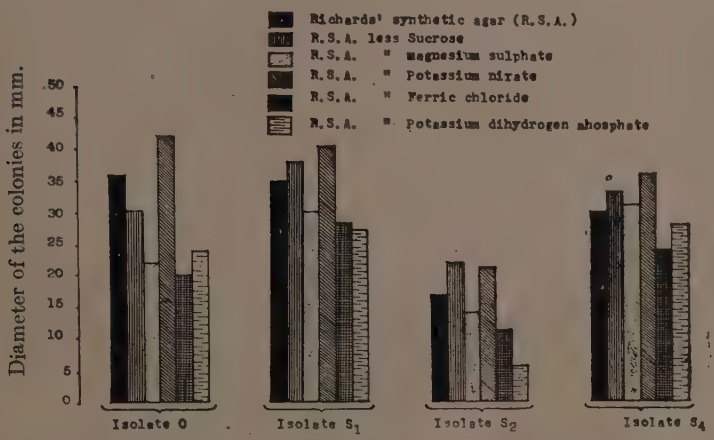

Fig. 3. Effect of Richards' synthetic agar medium and the media in which one of its components has been eliminated on the isolates of *S. rolfsii* and *O. texanum* var. *parasiticum*.

Summary

Sclerotium rolfsii Sacc. and *Ozonium texanum* Neal and Wester var. *parasiticum* Thirum. have been reported to cause severe damage to wide variety of hosts, wherever they occur. The two fungi though similar in their physiological and pathological responses, yet showed constant morphological differences of the cell size, sclerotial size and colour. Three

isolates selected out of isolates of *S. rolfsii* and an isolate of *O. texanum* var. *parasiticum* were taken up for detailed comparative studies. The different isolates of *S. rolfsii* maintained their individual character under varied cultural conditions; however, no pathological differences were observed among them. It was observed that on natural media the isolate of both the fungi produced a luxurient mycelial growth and a rich sclerotial crop, than on the synthetic or semi-synthetic media. They grew on a wide pH range of 3.2 to 9.2 with the optimum at pH 6.0; and the optimum temperature for growth being 30 to 34°C. Oxalic acid was freely produced by both the fungi and was found to be responsible for their toxicity to the host. The carbohydrate source in the medium did not seem to be readily assimilated by any of the isolates of the two fungi. The perpetuation of the two fungi appeared to be by means of sclerotia which could tolerate different weathering conditions. Factors responsible for their infection have been studied and the possible control measures have been indicated.

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A MOSAIC DISEASE OF SOYBEAN (GLYCINE MAX (L.) MERR.)

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During the routine survey of the economic crops grown in the Institute area, a mosaic disease of soybean (*Glycine max* (L) Merr) was observed in the month of September, 1956. All the 28 varieties including two exotic collections grown in the field were found to be affected with the disease. The incidence of the disease in different varieties varied from 7 to 30 per cent. Preliminary transmission tests indicated the disease to be of virus origin. An account of the disease together with the investigations conducted on the causal virus, its host range and mode of transmission are reported in the present paper.

Material and Methods. All experiments were conducted in an insect-proof glass house. The culture of the virus was obtained from naturally infected soybean plants and was maintained by successive transfers by mechanical inoculation to healthy seedlings. Mechanical inoculation was made by the usual leaf rubbing method using carborundum powder as an abrasive. The standard extract used in the thermal inactivation, longevity and ultraviolet irradiation tests was prepared by crushing young infected leaves to a fine pulp adding distilled water at the rate of one ml. per gram of leaf material. All the experiments were conducted with soybean plants of variety I.C. 211 which was observed to be the most susceptible under field conditions. For insect transmission te ts, healthy colonies of aphids collected from potato, cabbage, broad bean and chilli were raised separately in an insectary and these were tested for their virus transmitting ability. The effect of ultraviolet irradiation on the virus was studied by using an Alpine Sunlamp Model IX 220 Volts DC.

Field Symptoms. The symptoms of the disease as observed under field conditions are the mosaic mottling of the leaves accompanied by slight crinkling and reduction in size. The upper trifoliate leaves on main shoots and axillary growth of the diseased plants show typical mosaic symptoms. These leaves are slightly reduced in size and puckered with dark green puffy areas in the majority of cases. The leaves become distorted and narrowed than the normal with margins turning downwards as compared to the smooth surface of a healthy leaf lamina. The petioles and internodes are shortened to some extent. The symptoms on the older leaves are not so marked and they generally exhibit a mild mosaic mottling and later on remain more or less symptomless.

The mosaic affected plants are stunted in growth and set fewer pods. The pods are small and flattened, less pubescent than those on normal plants. The yield of seed is much reduced since a considerable proportion of the pods have no viable seeds and the remainder as a rule, contain not more than one or two seeds. Even these seeds are under sized. Fig. I

shows the symptoms of the disease on soybean leaves under field conditions.

TRANSMISSION OF THE DISEASE. The disease was successfully transmitted to healthy soybean plants by mechanical inoculation with the infective juice. Systemic mosaic symptoms were produced in about 7–10 days in the months of October and March. Symptoms appeared after 41–53 days when inoculations were carried out during the cold months i.e. December and January.

The first visible sign of the disease following mechanical inoculation of the primary leaves with the virus, appears as a yellowish vein clearing which develops in the minor veins of the developing trifoliate leaves. The veins of the affected leaves are usually transluscent when seen against light. This symptom is transitory and appears 7–10 days after inoculation. No symptoms are seen on the inoculated leaves. These symptoms further develop into mosaic mottling which consists of small, irregular, light green and dark green areas. The dark green areas are puffy and usually raised in the form of vesicles. Rugose symptoms usually develop on the second or third trifoliate leaf formed after inoculation and increase in severity on leaves developing subsequently. They become less pronounced as the plants become old. Fig. 2 shows the symptoms produced by artificial inoculation under insect-proof conditions.

HOST RANGE. In order to determine the host range of the soybean mosaic virus, 65 plant species belonging to 13 different families were mechanically inoculated with the virus. The plant species inoculated were as follows:

Leguminosae: Glycine max (L) Merr., *Phaseolus aureus* Roxb., *P. mungo* L., *P. trilobus* L., *P. lathyroides* L., *F. vulgaris* L. varieties Red Kidney, Giant Stringless and Plentiful, *P. aconitifolius* Jacq., *Crotalaria juncea* L., *Cyamopsis tetragonoloba* (L) Taub., *Arachis hypogaea* L., *Vicia faba* L., *Dolichos lablab* L., *D. biflorus* L., *Medicago sativa* L., *Melilotus alba* Desv., *M. indica* All., *Trigonella foenum-graecum* L., *Trifolium incarnatum* L., *Sesbania grandiflora* Pers., *Vigna sinensis* Savi., *V. sesquipedalis* (L) Fruwirth, *Cicer arietinum* L., *Pisum sativum* L., *Lathyrus odoratus* L., *Cajanus cajan* (L) Millsp.

Cruciferae: Brassica oleracea L. var. *capitata* L., *B. caulorapa.*, Pasq., *B. rapa* L., *B. campestris* L. var. *dichotoma*, *B. campestris* L. var. *sarson* Prain, *B. campestris* L. var. *toria* Duthie and Fuller, *B. nigra* L., *B. juncea* L., *Raphanus sativus* L.

Compositae: Lactuca sativa L. *Carthamus tinctorius* L., *Zinnia elegans* Jacq., *Helianthus annus* L., *Tagetes patula* L.

Apocynaceae: Vinca rosea L.

Chenopodiaceae: Spinacea oleracea L., *Beta vulgaris* L.

Umbelliferae: Apium graveolens L., *Daucus carota* L.

Euphorbiaceae: Ricinus communis L.

Amaranthaceae: Amaranthus spinosus L., *Gomphrena globosa* L.

Solanaceae: Lycopersicon esculentum Mill, *Capsicum frutescens* L., *Datura stramonium* L., *Nicotiana tabacum* L. varieties *Harrison Special* and *White Burley*, *N. glutinosa* L., *Solanum nigrum* L., *S. nodiflorum* Jacq., *S. melongena* L., *S. tuberosum* L. varieties *President*, *Uptodate* and *Craig's defiance*.

Linaceae: Linum usitatissimum L.

Cucurbitaceae: Lagenaria siceraria Standl., *Citrullus vulgaris* Schrad., *Cucumis sativus* L., *Momordica charantia* L., *Trichosanthes anguina* L.

Malvaceae: Gossypium arboreum L., *Abelmoschus esculentus* (L) Moench.

Pedalliaceae: Sesamum indicum DC.

Except soybean, none of the plant species listed above produced any symptoms of disease and it was confirmed by back inoculating that they were not carrying the virus symptomlessly showing thereby that the virus under study has a very restricted host range.

Physical properties. The virus in the standard extract was found to be infective when heated to 60 °C. for 10 minutes but was rendered innocuous when exposed to 62 °C. for the same period. It could withstand a dilution of 1 : 1000 but the infectivity was lost when diluted to 1 : 5000. The standard extract of the virus was infective after storage for 3 days at room temperature (15–22 °C) but not after 4 days. At frigidaire temperature (7–10 °C), the virus could withstand storage for 5 days but not for 6 days. The virus was found to be viable on desiccation of the detached mosaic affected leaves for 7 days at room temperature (25–33 °C) but was inactivated after 8 days. Exposure for two hours to ultraviolet rays irradiated from a distance of 24 inches by an Alpine Sunlamp Model IX running on 220 Volts DC completely inactivated the virus in the leaf extract, but an exposure for one hour or less had no effect on its infectivity.

Insect transmission tests. Four species of aphids were collected from different crops such as *Myzus persicae* Sulz. from potato, *Lipaphis erysimi* Kalt from cabbage, *Aphis craccivora* Koch from broad bean and *Aphis gossypii* Glov. from chilli and their colonies were raised in insectary on tobacco, cabbage, cluster bean and chilli respectively.

Individual groups of aphids from above colonies were collected and starved for about 1 to 4 hours before feeding on diseased plants. The insects were allowed to feed on the diseased plants for a period of 2 to 24 hours after which they were removed and liberated on healthy soybean test plants on which they were allowed to remain for a period of about 24 to 48 hours. The insects were killed by spraying with 0.1 per cent Ekatox solution after the feeding period was over. The test plants were kept

under observation for a period of six weeks after which they were discarded. The results of these tests are given in Table I.

TABLE I. Insect transmission of Soybean mosaic virus

Insect species tested	Date of experiment	No. of insects on each test plant	Period of			Number of plants	
			Starvation in hrs.	Feeding on diseased plant in hrs.	Feeding on healthy test plants in hrs.	Tested	Infected
1. *Myzus persicae* Sulz.	9.3.57	15	1	3	24	7	4
	19.3.57	12	3	3	48	7	6
	27.3.57	15	4	24	48	12	7
2. *Lipaphis erysimi* Kalt.	11.3.57	15	1	2	24	8	5
	28.3.57	20	2	24	48	11	7
3. *Aphis craccivora* Koch	12.3.57	15	1	4	24	6	4
	28.3.57	20	2	24	48	18	15
4. *Aphis gossypii* Glov.	11.3.57	15	2	4	48	6	3

The data presented in Table I show that all the four species of aphids tested namely *Myzus persicae* Sulz., *Lipaphis erysimi* Kalt., *Aphis craccivora* Koch and *Aphis gossypii* Glov. are able to transmit the soybean mosaic virus.

SEED TRANSMISSION TRIALS. In order to determine whether or not the disease under study was seed borne, seeds were collected exclusively from mosaic affected plants of soybean (I.C. 210 and I.C. 211) grown in the field as well as in the glass house. These seeds were sown in the glass house nursery in 10 inch pots, each having about 25 seeds. The seedlings raised from these were kept under observation for a period of about 8–10 weeks after which they were discarded. The number of plants showing typical mosaic symptoms of the disease were recorded. The results are presented in Table II.

The seed transmission trials (Table II) indicate that the soybean mosaic virus is transmissible through the seed collected from the diseased plants and the percentage of seed transmission in different lots varied from 2.7 to 7.9.

TABLE II. Seed transmission trials

Date of collection	Source of seed	Variety	Date of sowing	Number of seeds sown	Number of seeds Germinated	No. of plants showing infection	Per cent of seed transmission
5.12.56	Field	I.C.211	10.4.57	310	257	17	6.6
5.12.56	Field	I.C.210	12.4.57	250	179	5	2.8
15.2.57	Glass house	I.C.211	9.4.57	48	35	1	2.9
27.2.57	—do—	—do—	9.4.57	53	42	2	4.8
1.3.57	—do—	—do—	—do—	45	37	1	2.7
15.3.57	—do—	—do—	—do—	50	39	2	5.1
29.3.57	—do—	—do—	—do—	47	38	3	7.9

DISCUSSION. Although a sap transmissible mosaic disease of soybean has been reported from several countries, detailed studies on the causal virus have been conducted by only a few workers viz. Gardner and Kendrick (1921, 1924) from Indiana (U.S.A.), Pierce (1935) from Wisconsin (U.S.A.), Heinze and Kohler (1940) from Germany and Conover (1948) from Illinois (U.S.A.). Gardner and Kendrick (1921, 1924) did not study the physical properties of the virus but reported that the virus was transmitted by sap inoculation and through seed. Their attempts to transmit the disease to 60 varieties of garden beans, 7 species of *Phaseolus*, 2 species of *Dolichos*, field peas and cowpeas gave negative results. Pierce (1935) reported that the soybean mosaic virus had a thermal-death-point of 56–58 °C. and longevity *in vitro* of 3–4 days. The virus was found to be transmitted to soybean only and no infection was obtained on any other plant species tested. Heinze and Kohler (1940) from their study of the soybean mosaic virus occurring in Germany reported that the lethal temperature for the virus in sap was 61 °C and in the unpurified sap the virus remained viable for 3–4 days at room temperature (21–23 °C). The virus was found to be seed transmitted and 8 species of aphids viz. *Aphis fraugulae*, *A. rahmni*, *A. fabae*, *Macrosiphum solanifolii*, *M. solani*, *Myzus ornatus*, *M. circumflexus* and *M. persicae* proved to be the vectors. Their attempts to transmit the virus to garden and field peas and *Vicia villosa* gave negative results. Conover (1948) isolated two different viruses from mosaic affected soybean plants and identified them to be *Soja virus* 1 and *Phaseolus virus* 2. The *Soja virus* 1 had a thermal inactivation point of 64–66 C and a longevity *in vitro* of 4–5 days at room temperature (18.5 °C). The virus could not be transmitted to any of the 23 plant species tested but was recovered from the inoculated primary leaves of certain varieties of *Phaseolus vulgaris*. The virus was found to be seed transmitted as also by *Myzus persicae* and *Macrosiphum pisi*. The causal virus in all the above cases has been designated as the *Soja virus* I or Soybean virus 1 (Smith 1957). The virus reported in the present paper also resembles closely the above in symptomatology, host range, physical properties, seed transmission and having aphids as its vectors. The soybean mosaic reported herein is, therefore, caused by *Soja virus* I, Smith or Soybean virus I, Pierce.

Fig. 1. Healthy and mosaic affected leaves of soybean.

Fig. 2. Soybean plant infected by sap inoculation.

Summary

A sap transmissible virus disease of soybean characterised by a mosaic pattern of irregular light and dark green areas on the leaves and accompanied by blistering and deformation of the leaf lamina has been described.

The host range of the virus is found to be restricted to soybean only, none of the other 64 plant species tested being infected with the virus. The causal virus has a thermal death point of 60–62 °C, a dilution-end-point between 1 : 1000 and 1: 5000 and longevity *in vitro* of 3–4 days at room temperature (15–22 °C) and 5–6 days at frigidaire temperature (7–10 °C). The virus is inactivated on desiccation of the infected leaves at room temperature (25–33 °C) for 8 days and after 2 hours' exposure to ultraviolet rays.

Four species of aphids viz. *Myzus persicae* Sulz., *Aphis gossypii* Glov., *A. craccivora* Koch. and *Lipaphis erysimi* Kalt proved to be the vectors of the virus. The disease was also found to be transmitted through the seed of diseased plants, the percentage of seed transmission varying from 2.7 to 7.9 in different lots.

The causal virus has been identified as *Soja virus* 1 (Smith, 1957) or Soybean virus I (Pierce, 1935).

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STUDIES ON BLIGHT DISEASE OF MANGO CAUSED BY MACROPHOMA MANGIFERAE

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Introduction: Mango (*Mangifera indica L.*) has been known to the people of India since very early days and has been under cultivation for more than 4,000 years. It has been estimated that nearly, 80,00,000 tons of mango fruit are produced annually and that the total area under cultivation is about 3 million acres. It is grown almost throughout India up to an elevation of 4,000 feet.

Mango plants are subject to several fungal and bacterial diseases as also to physiological disorders, which take a heavy toll of the yield every year. A species of *Macrophoma*, identified as *M. mangiferae* sp. nov. by Hingorani and Sharma (1956), was observed on mango leaves in September, 1949, by the Senior author in the orchard of Entomology Division, Indian Agricultural Research Institute, New Delhi, which produced irregular, brown-coloured spots having raised dark purplish margins with black pycnidial bodies on the necrotic area of the leaf. The disease was subsequently found to be present throughout the year in all parts of Delhi State, but heavy infection was observed during the monsoon season i.e. July to September.

Prior to this, Averna Lacca (1922) from Brazil recorded a species of *Macrophoma* causing black rot of mango fruits on trees; and Patel, Kamat and Bhide (1949) described a species of *Macrophoma* on branches and fruits of mango from India which had been earlier considered as a species of *Phoma* by Kanitkar and Uppal (1939).

Since there was only one previous record of *Macrophoma* sp. on mango from India and that also on stems and fruits but not on leaves, it was considered worthwhile to study the disease and its pathogen in detail and the results so obtained are recorded in this paper.

The symptoms of the disease given below are those observed in nature under Delhi conditions.

The disease usually appears as yellowish pin-head like spots on leaves and twigs of the affected plants. These gradually enlarge discolouring the surrounded tissue, which first become light brown and then drak brown, with slightly raised and broad dark purplish margins, and later ashy coloured due to the appearance of pycnidia. Spots are round at first, but later become oval or irregular, the size depending upon the environmental conditions such as humidity and temperature. The spores that are washed down by rains accumulate in droplets at the tip of a leaf, which dries up entirely, and the infection travels downward toward the petiole. Sometimes half or more than half of a leaf may become involved.

Pycnidia make their appearance mostly on the under surface of the leaves, but sometimes on the upper surface too, as black dots scattered all over the necrotic portion. Dots are minute, mostly single and innumerable. Infection is observed mainly on leaves (Fig. 1) and rarely on stems. On stems, the lesions are elliptic which later girdle the stem at the point of infection. On fruit, water-soaked, circular lesions are produced which enlarge rapidly and cause rot, particularly in storage. In field, however, fruit infection is rare.

EXPERIMENTAL: (a) ISOLATION OF THE PATHOGEN: About 100 isolations were made from the diseased leaves and twigs of mango, collected from different orchards in Delhi State, and in all cases a species of *Macrophoma* was obtained. The cultures were single-spored and, since they were identical in their major characters, one of them was selected for detailed study.

(b) INOCULATION TESTS: In order to prove the pathogenic nature of the fungus isolated, young potted plants of mango varieties *Langra*, *Chowsa* and *Dashari* (average height of the plants about 2 ft. and age 6 months) were first kept in moist chambers for 24 hours before inoculation. Leaves were surface sterilized with rectified spirit and washed with sterilized water. A small piece of the inoculum, consisting of spores and mycelium of the fungus, was placed on injured and uninjured surfaces of the leaves. The injury was made either with a very fine pin or by rubbing with carborundum. The inoculum was then covered with a piece of moist cotton wool. The inoculated plants were placed inside glass cages in which humidity was maintained to near saturation point for 48 hours by means of frequent sprays of water with the help of a pressure sprayer. They were then kept outside on the branch for observation. Control plants received the same treatment as the inoculated ones except that no inoculum was used. Average maximum and minimum temperature recorded during the experimental period were 33°C and 11°C., respectively. Fresh healthy fruits and green tender twigs of mango were also inoculated with and without injury and placed in moist chambers for 7 days. Typical symptoms of the disease appeared on the injured leaves and twigs of all the three varieties. Also the fruits, both immature and mature, developed infection. In the early stages of infection, yellowish spots were formed at the point of inoculation after 3 to 4 days. These spots later became irregular and dark brown in colour. Within 10 days, black pycnidia (Fig. 2) were formed on the opposite side of the inoculated surface and the symptoms resembled those found in nature. The reisolation made from the artifically inoculated leaves, twigs and fruits always yielded a species of *Macrophoma*, identical with the parent culture.

Ficus carica, *Eryobotrya japonica*, *Eugenia jambolina*, *Vitis vinifera*, *Psidium guajava*, *Nicotiana tabacum*, *Musa sapientum*, *Citrus sinensis*, *Cucumis sativus*, *Physalis peruviana*, *Zea mays and Cajanus cajan* were also inoculated after injury and the first four hosts became infected.

(c) MORPHOLOGY: Morphological characters of the pathogen were studied on the host and from a 2-month old culture grown on oatmeal agar.

On the host, sections through the necrotic portion show hyaline septate mycelium in all the tissues. The mycelium is both inter-and intra cellular. The affected cells turn brownish, then become devoid of chloroplasts and lose their shape. Pycnidia are found on the necrotic portions of the spots. They are globose or sub-globose, light brown, ostiolate, deep seated in leaf tissue, subepidermal, at first innate and then erumpent, measuring 77–231 μ in diameter. Pycnidial wall is parenchymatous and 5–6 layers thick. Conidiophores are slender, hyaline, 8–11 μ x 1.5–2 μ in size, bearing conidia singly on tips. Conidia are single-celled, hyaline, oblong elliptical with both ends rounded, slightly broader in the middle with granular contents and one to three oil drops. These conidia measure 10.5–24.5 μ x 5.3–7 μ (average 19.8 μ x 6.5 μ).

On oatmeal agar, the mycelium is at first spreading but shortly develops abundant white, cottony growth. Aerial hyphae are hyaline, septate, slender, measuring 1.8–7 μ in diameter, with granular protoplasmic content. The colour of the mycelium changes to olive green with age. The submerged mycelium is much branched, stout with oil globules, at first hyaline and then becomes dark olive and thick walled after 3–4 days of growth, imparting colour to the substratum. Pycnidia are formed as small black specks enclosed in loose hyphal wefts of the aerial mycelium on the surface of the medium. Pycnidia are black, sclerotic with hard pycnidial wall; measure 225–510 μ x 210–450 μ (average 309 μ x 278.9 μ) and are ostiolate, ostiole appearing as a small depression. Cream coloured, mucilaginous conidial mass oozes from the ostiole and collects there as droplets. The sporophores are thin, slender, cylindrical, hyaline, slightly broader at the tips and measure 8.8–24.4 μ x 1.8–3.5 μ (average 16.2 μ x 2.6 μ). The spores are hyaline with granular protoplasmic content, single celled, oblong to elliptical with both ends rounded and slightly broader in the middle, measuring 17.5–29.8 μ x 3.5–7.5 μ (average 23.1 μ x 5.6 μ). (Fig. 3).

The technical description and Latin diagnosis of the pathogen have been provided in an earlier paper (Hingorani and Sharma, 1956).

(d) PHYSIOLOGY: Spores of the pathogen germinate equally well in tap and distilled water. Spores from a 2-month old culture germinate in about 2¼ hours at 26 °C., while those from the host germinate within 3 hours. In the former case, 100 per cent germination is never obtained, while in the latter case all the spores germinate within 24 hours. The germ tubes are long, granular, septate and branched. Branching is more pronounced at low temperatures (7–10 °C) when the rate of growth is very slow. Septa are formed just before germination of spores. Spores do not germinate unless a film of water is present, but they germinate at a wide range of temperatures (10 °C–35 °C.), maximum germination taking place between 25 °C–35 °C. and none at 5 °C and 40 °C. If the pycnidia are kept in water, spore masses exude through ostioles, after 5 minutes.

The fungus grows well on potato-dextrose agar, oatmeal agar, Czapeck's medium and corn-steep liquor, but not on mango-leaf extract, onion extract and carrot-extract agar. On most of these media pycnidia

are not formed, but on oat-meal agar and Brown's agar medium a few mature pycnidia are produced in about forty days.

On potato dextrose agar the fungus covers the Petri plate (96 mm.) completely within 4 days forming a regular and circular colony. The aerial mycelium is hyaline, cottony, slender, granular, distantly septate and branched. The submerged mycelium is hyaline at first, but changes to olive or dark olive within 3 days. It comprises both stout and slender branched hyphae, with granular contents and thick walls, which form a thick mat. Colour of the substrate is at first straw yellow and then it changes to dark olive. The temperature range for its growth is wide (7 °C–35 °C.,) the minimum, the optimum and the maximum temperatures being 7 °, 25–35 ° and 40 °C., respectively. Thermal-death-point of the fungus lies between 50 ° and 55 °C. The optimum pH range for the growth of the fungus is 4.6–6.4, the minimum being 2.4–3.6 and the maximum 7.0 to 8.0. Pycnidia are not formed.

Effect of different carbon sources (sucrose, glucose, lactose fructose, maltose and arabinose), nitrogen sources (asparagin, ammonium tartrate, ammonium nitrate and glycine) and vitamins (thiamine, biotin, pyridoxine and inositol) on sporulation of the fungus was also studied. Glucose, lactose and fructose promoted pycnidial formation slightly, but fewer mature pycnidia were formed on these sugars than on oatmeal agar. None of the other substances tried had any appreciable effect on sporulation.

(e) Effect of environmental factors on disease development: The effect of temperature on the intensity of infection was studied by inoculating the mango plants of a susceptible variety *Dashari* during the months of March, June and December when the temperature varied considerably. During the month of June, when the temperature varied from 27 °C to 41 °C., 100 per cent infection was obtained. During December, when the average maximum temperature was 25 C. and the minimum 9 °C., the disease did not develop.

In order to study the influence of moisture on the intensity of the disease, inoculated plants were kept for different periods in humid chambers in which humidity was maintained to almost a saturation point by frequent sprays with a hand pressure sprayer. After a fixed time for a particular set of the inoculated plants, the pots were shifted to glass cages where no humidity was provided. During the experimental period, the average maximum temperature recorded in glass cages was 35 °C. and the minimum 21 °C. No infection took place when the period of saturated humidity was less than 40 hours, and 100 per cent infection was achieved when the period was 72 hours. In nature typical symptoms of the disease with abundant pycnidial formation occurred during the period July to September, when high temperature and humidity prevailed.

(f) Perpetuation of the pathogen: Viability of the pathogen under field conditions was tested by placing the infected leaves at different depths of soil (0, 2, 4 and 6 inches) in pots which were then kept in the open. Isolations were made from this material every month, as also from

the fallen leaves which had been shed by the trees. The pathogen was found to be viable even after one year. It was also isolated from one year-old dried material available in the herbarium.

(g) CONTROL OF THE DISEASE: The effect of Burgundy mixture, Perenox, Lime sulphur, and Dithane on spore germination of the pathogen was tested in the laboratory.

Burgundy mixture of 2 per cent, 1 per cent and 0.5 per cent strength was sprayed on the slides by Pitter's sprayer at the rate of 2 cc. per slide. Spore suspension was put on the slides and the percentage of spore germination after 24 hours was found to be 0,15 and 49 in 2 per cent, 1 per cent and 0.5 per cent Burgundy mixture, respectively. Likewise, 0, 24 and 56 per cent spore germination took place in $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ per cent Perenox, respectively.

The stock solution of Lime sulphur was prepared in the proportion of 4 : 8 : 80 and then diluted from 100 to 600 times. One c.c. of spore suspension, with a heavy spore load, was added to 4 cc. of the solution and spore germination was tested on a slide. The percentage of spore germination was found to be 2, 8, 18, 24, 37 and 50 at the dilution of 1 : 100, 1 : 200, 1 : 300, 1 : 400, 1 : 500 and 1 : 600, respectively. Using the same technique, dilution of 1 : 9000 of Dithane completely inhibited spore germination.

Thus, spores of the pathogen did not germinate in 2 per cent Burgundy mixture, $\frac{1}{2}$ per cent Perenox, and 1 : 9000 dilution of Dithane.

Although experiments for the control of the disease have not been undertaken, evidence obtained in spore germination tests has shown that the fungus is sensitive to low concentrations of some of the fungicides stated above, indicating thereby that these fungicides may be profitably utilized for the control of the disease. Since the pathogen survives in the leaves, it will also be helpful if the infected leaves are collected and burnt.

SUMMARY

A blight disease of mango, caused by *Macrophoma mangiferae*, has been described. Besides *Mangifera indica*, the pathogen weakly infects *Eryobotrya japonica*, *Eugenia jambolina*, *Ficus carica* and *Vitis vinifera*.

The pathogen can survive for more than a year on leaves of mango.

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Fig. 1. Diseased leaves of mango showing blight symptoms in nature.

Fig. 2. Diseased portion of a leaf enlarged, showing pycnidial structures after artificial inoculation.

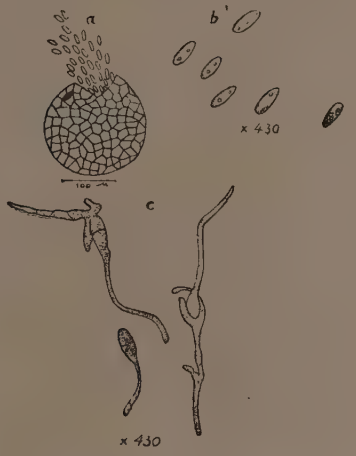

Fig. 3. a. Pycnidium and pycnidiospores from the host;
b. Spores;
c. Germinating spores;

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SOME CERCOSPORA SPECIES FROM INDIA-IV

R. L. Munjal, G. Lall and B. L. Chona

(Accepted for publication May 10, 1960)

In this paper, we have recorded eleven species of *Cercospora*, two of which are new to science and the rest either new records or new host records for India. The first three papers of the series have been published in Indian Phytopathology Vol. **12**, pp. 76-84; 85-89, 131-138, 1959. The specimens have been deposited in Herbarium Cryptogamiae Indiae Orientalis and their numbers indicated in the text.

Cercospora agnostoica Speg., Rev. Mus. La Plata. **15** : 45, 1908; Sacc. XXII : 1426, 1913.

On living leaves of *Symphytum* sp. (Boraginaceae), I.A.R.I., New Delhi (Delhi), 5–9–1959, G. Lall, H.C.I.O. No. 26623.

The fungus produces definite spots on leaves which are tan coloured and some are ash coloured in centre, bearing fructifications mostly on the under surface of the leaf. The conidiophores arise from small stromata in fascicles, are pale brown, not branched, sparingly septate and geniculate, and measure 3.8–5.8 x 15.4–50.0 μ. The conidia are acicular, hyaline, septate and measure 2.9–3.8 x 30.8–138.6 μ.

Cercospora atylosiae Thirum. & Govindu, Sydowia **7** : 311, 1953.

On living leaves of *Atylosia lineata* W. & A. (Leguminosae), I.A.R.I., New Delhi (Delhi), 25–9–1958, G. Lall, H.C.I.O. No., 26622.

On the leaves, the fungus forms spots which are brown to black, diffuse and coalescent, bearing fructifications on the upper surface of the leaf. The conidiophores arise from well developed stromata in dense fascicles which are pale brown, not branched, rarely septate and measure 3.8–4.8 x 17.3–86.3 μ. The conidia are hyaline, acicular, septate and measure 2.9–3.8 x 38.5 – 123.2 μ.

Thirumalachar & Govindu (l.c.) reported the above species from South India on *Atylosia scarabaeoides* Benth.

Cercospora bombacicola sp. nov.

Foliorum maculae subcirculares ad angulares, dispersae vel non-numquam confluentes, 1–15 mm. diam., griseo-albae vel bubalinae in medio, marginibus fusce brunneis; fructificationes ut plurimum in pagina superiore foliorum; stromata constantia cellulis paucis brunneis usque ad 42.5 μ diam. subglobosa, fusce brunnea; fasciculi pauci vel plurimi;

conidiophori olivaceo-brunnei, apicibus pallidioribus, septati, varie curvati, plures geniculati, irregulares latitudine, rarissime ramosi, sporarum cicatricibus eminentibus, 4–6 x 39–150 μ; conidia hyaline, acicularia, indistincte multi-septata, truncata ad basim, acute vel subacuta ad apicem, 2 – 4 x 46 – 150 μ.

In foliis viventibus *Salmaliae malabaricae* Sch. & End. e familia Bombacacearum, ad Chamba in H.P. die 7 novembris anni 1958, H. S. Gill, H.C.I.O. No. 26626.

Cercospora bombacicola sp. nov.

Leaf-spots subcircular to angular, scattered, or sometimes confluent, 1–15 mm. in diameter, greyish-white to buff centre with dark brown margin; fruiting mostly on the upper side of the leaf; stromata from few brown cells to 42.5 μ. in diameter, subglobose dark brown; fascicles few to dense; conidiophores olivaceous brown, tip dilutely coloured, septate, variously curved, many geniculate, irregular in width, very rarely branched, spore-scar prominent, 4–6 x 39–150 μ; conidia hyaline, acicular, indistinctly multiseptate, base truncate, tip acute to subacute, 2–4 x 46–150 μ.

On living leaves of *Salmalia malabarica* Schott. & Endl., (*Bombax malabaricum* DC.) (Bombaceae), Chamba (H.P.), 7–11–1958, H. S. Gill, H.C.I.O., No. 26626.

Cercospora brassicicola P. Henn., Bot. Jahrbuecher v. Engler **37** : 166, 1906; Sacc. XXII : 1413, 1913.

On living leaves of *Brassica juncea* Coss., Shri Pasang Twang's Garden, Kalimpong (West Bengal), 25–10–1956, S. P. Raychaudhury, H.C.I.O. No. 26624; of *B. oleracea* L. var. *botrytis* . (Cruciferae), I.A.R.I., New Delhi (Delhi), 26–9–1959, Ved Prakash and Gian Singh, H.C.I.O. No. 26631.

The fungus produces definite spots on leaves which are yellowish grey or white with brown margin, bearing fructifications on both surfaces of the leaf. The conidiophores come out of the stromata in fascicles, which are olivaceous brown, curved or undulate, septate, geniculate, and measure 2.9–3.8 x 38.5–134.7 μ in the case of *Brassica juncea* and 2.0–3.8 x 30.8–365.8 μ in the case of *B. oleracea* var. *botrytis*.

Cercospora canescens Ell. & Mart., Amer. Nat. **16** : 1003, 1882; Sacc. IV : 435, 1886.

On living leaves of *Phaseolus aureus* Roxb., I.A.R.I., New Delhi (Delhi), 12–9–1959, Ved Prakash & Gian Singh, H.C.I.O., No. 26628; of *P. radiatus* L. (Leguminosae), Development Area, Kalimpong (West Bengal), 9–11–1956, S. P. Raychaudhuri, H.C.I.O., No. 26629.

The fungus produces definite spots on leaves which are at first brown, later turning grey or dirty grey with narrow reddish brown margin bearing

fructifications on both the surfaces. The conidiophores come out from stromata in dense fascicles which are olivaceous brown to dark, septate, geniculate, and measure 3.8–5.8 x 19.3–130.9 μ. The conidia are hyaline, acicular, septate, and measure 2.9–4.8 x 73.2–231.0 μ.

This species has already been reported on *Vigna catjang* by Thirumalachar & Chupp (Mycologia **40** : 354, 1948) and on *Phaseolus trilobus* by Thirumalachar & Govindu (Sydowia **10** : 259, 1956) from India.

Cercospora commonsii Sacc., Syll. Fung. X : 623, 1892.

On living leaves of *Stylosanthes gracilis* H. B. & K. (Leguminosae), I.A.R.I., New Delhi (Delhi), 25–9–1958, G. Lall, H.C.I.O., No. 26620.

The fungus produces small to large spots on leaves which are dark brown bearing fructifications on both the surfaces of the leaf. The conidiophores are pale olivaceous brown, simple, septate, rarely geniculate, and measure 3.8–4.8 x 38.5–161.7 μ. The conidia are hyaline, acicular to narrowly obclavate, septate and measure 2.9–3.8 x 46.2–115.5 μ.

Cercospora latens Ell. & Ev., Jour. Mycol. **4** : 3, 1888; Sacc. X : 641, 1892.

On living leaves of *Lespedeza instanica*, L. *stipulacea* Maxim (= *L. striata* Hook. & Arn.), (Leguminosae), I.A.R.I. New Delhi (Delhi), 25–9–1958, G. Lall, H.C.I.O., No. 26617 & 26618 respectively.

This species has already been described on *Psoralea corylifolia* by Munjal, Lall & Chona (Indian Phytopath. **12** : 86, 1959) from India, and the present fungus on the above two hosts show stromata up to 30.4 μ in diameter and conidiophores measuring 3.8–5.8 x 10.5–50.1 μ and conidia measuring 3.8 x 19.3–57.8 μ.

Cercospora menthicola Tehon & Daniels, Mycologia **17** : 247, 1925.

On living leaves of *Mentha sylvestris* Linn. var. *incana* (Labiatae), I.A.R.I., New Delhi (Delhi), 25–9–1958, G. Lall, H.C.I.O., No. 26621.

On leaves, the fungus forms spots which are gray with dark purple margin, bearing fructifications on both the surfaces of the leaf. The conidiophores arise from a small stromata in spreading fascicles which are olivaceous brown, not branched, septate, geniculate, and measure 3.8–4.8 x 38.5–115.5μ. The conidia are hyaline, acicular, septate, and measure 2.0–3.8 x 38.5–136.2μ.

Cercospora ocimicola Petrak & Cif., Annal. Mycol. **30** : 324, 1932.

On living leaves of *Ocimum kilimandscharium* Guerke (Labiatae), I.A.R.I., New Delhi (Delhi), 25–9–1958, G. Lall, H.C.I.O., No. 26619.

The fungus at first produces yellowish discolourations on the upper surface of the leaf, later on greyish brown areas are formed, bearing fructi-

fications chiefly on the lower surfaces. The conidiophores come out of the stromata in fascicles which are pale olivaceous brown, septate, and measure 3.8–4.8 x 26.9–94.2 μ. The conidia are pale, cylindric, septate, and measure 3.8 x 15.4–50.0 μ.

Cercospora paludicola Speg., Anal. Soc. Cient, Argentina 13 : 29, 1882; Sacc. IV: 455, 1886.

On living leaves of *Polygonum* sp. (Polygonaceae), Solan, Simla Hills (Punjab), 5–8–1959, P. C. Joshi, H.C.I.O., No. 26625.

The fungus produces minute spots on leaves which are pale to dark brown with tan coloured border, bearing fructifications on the undersurface of the leaf. The conidiophores come out in fascicles which are pale, brown, short, aseptate, without geniculations, and measure 3.8 x 7.7–26.9 μ.

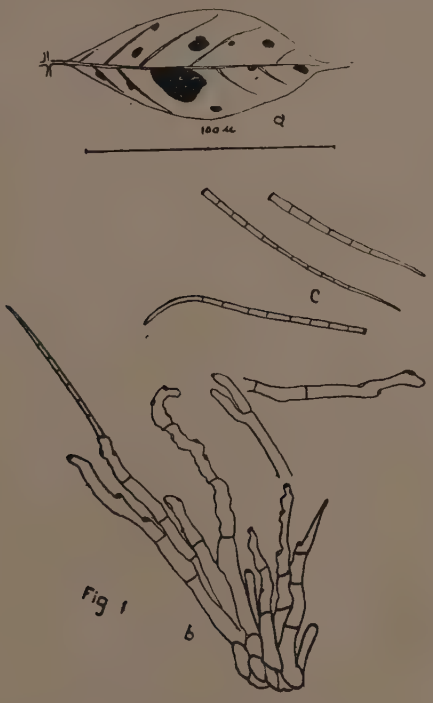

Fig. 1. *Cercospora bombacicola*
a. Leaf showing spots (Diagrammatic)
b. Stroma with conidiophores
c. Conidia.

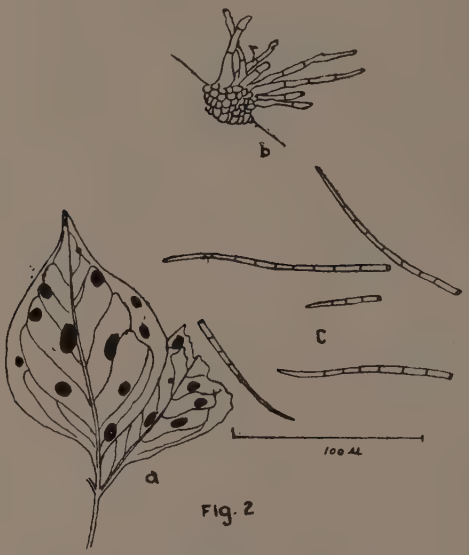

Fig. 2. *Cercospora typhonii*
a. Leaf showing spots (Diagrammatic).
b. Fascicle of conidiophores
c. Conidia.

The conidia are hyaline to subhyaline, narrowly obclavate, septate, and measure 2.9–3.8 x 26.9–84.7µ.

Cercospora typhonii sp. nov.

Maculae circulares vel subcirculares, dispersae vel confluentes, 2–9 mm. diam., luteolo-virides vel aerugineae in medio, margine lato luteolo, haud definito; fructificationes amphigenae, sed frequentiores in pagina superiore foliorum; stromata vel paucis cellulis constantia vel usque ad 26.9 µ diam., subglobosa, fusce brunnea; fasciculi pauci vel densi; conidiophori pallide vel olivaceo-brunnei, apicibus pallidioribus, septati, vix geniculati, irregulærea latitudine, non ramosi, supra attenuati, 4–6 x 15–85 µ; conidia hyalina, acicularia, septata, recta vel curvata, truncata ad basim, subacuta ad apicem, 3–4 x 23–131 µ.

In foliis viventibus *Typhonii trilobati* Schott. e familia Aracearum, ad Calcutta in Bengalia occidentali, 2 novembris anni 1939 A. K. Ghosh, H.C.I.O. No. 26616.

Cercospora typhonii sp. nov.

Leaf-spots circular to subcircular, scattered or confluent, 2–9 mm. in diameter, yellowish-green to verdigris in centre with broad yellowish, indefinite margin; fruiting amphigenous but more on the upper side of

the leaf; stromata from few cells to 26.9 μ in diameter, subglobose, dark brown; fascicles few to dense; conidiophores pale to olivaceous brown, tip dilutely coloured, septate, sparingly geniculate, irregular in width, not branched, attenuated above, 4–6 x 15–85 μ; conidia hyaline, acicular, septate, straight to curved, base truncate, tip subacute, 3–4 x 23–131 μ.

On living leaves of *Typhonium trilobatum* Schott (Araceae), Calcutta (West Bengal), 2–11–1939, A. K. Ghosh, H.C.I.O. No. 26616.

Earlier a *Cercospora* sp. has been reported on the above host from Bengal by Roy (Fungi of Bengal. Bull. Bot. Soc., Bengal **2** : 134–177, 1948) but he did not make the specific determination.

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest, encouragement and valuable criticism. We are also indebted to Rev. Fr. Dr. H. Santapau, Head of the Biology Department, St. Xavier's College, Bombay for rendering the latin diagnosis of new species and to Mr. J. N. Kapoor, Herbarium Keeper for help in the identification of some species.

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A COMMONLY OCCURRING LEAF SPOT DISEASE CAUSED BY MYROTHECIUM RORIDUM TODE ex FR.

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Introduction: During the last few years a disease has been observed at Delhi which affects a variety of economic crops and does great damage to the foliage. The plants chiefly affected are cotton (*Gossypium hirsutum, G. herbaceum* & *G. indicum*); *Cucurbits* like *Lagenaria siceraria, Citrullus vulgaris, Cucurbita moschata, Momordica charantia* and *Trichosanthes anguina*; certain vegetable and pulse crops such as *Abelmoschus esculentus, Vigna sinensis, Phaseolus aureus* and *Cyamopsis tetragonoloba*; and Jute (*Corchorus capsularis*). Earlier it was recorded on *Vigna sinensis* (Padmanabhan, 1946), *Hibiscus esculentus* (Thirumalachar and Mishra, 1953) and *Cyamopsis tetragonoloba* (Arya, 1956).

The disease has also been reported to occur in Africa. At Sierra Leone it affects *Luffa acutangula, Abelmoschus esculentus, Trichosanthes anguina* and *Corchorus olitarius* (Deighton, 1936) while in Belgian Congo it attacks cotton (Steyaert, 1948). In Europe and U.S.A., the usual leaf-spot symptoms are not observed but the fungus causes stem rot, root rot and fruit rot. Preston (1936 & 1943) has reported a serious stem and root rot disease of Pansies and Violas from England while Taubenhaus (1935) and Stevenson & McColloch (1947) have recorded crown rot of green house Snapdragons and fruit rot of tomato respectively from the United States.

Symptoms: The disease in Delhi appears during the rainy season as small, circular, tan coloured spots with broad violet to brown margin surrounded by zones of translucent areas which give it the appearance of concentric rings. Later, on these translucent areas the fructifications of the fungus appear as dark green sporodochia surrounded by a rim of white hair like mycelia, which become conical or flattened. Generally the incidence of the disease is low. In severe cases, 5–10 per cent of the leaf area may be destroyed. Sometimes the spots may coalesce and the entire leaf may dry up, giving the plant a wilted appearance.

Experimental (a) Isolation: Isolations were made from the infected spots, which were surface sterilized with 0.1 per cent mercuric chloride solution, washed in sterile water and then transferred to 2 per cent acidified plain agar slants, as also by transferring sporodochia directly from the host to the medium. Numerous colonies of the fungus bearing spores came up. Spores and mycelium from these were again transferred to the P.D.A. There was no difference in the growth, fructifications or spores of isolates so obtained. These were further purified by single spore technique and maintained on P.D.A. at room temperature (22–27° C).

(b) Morphology: The mycelium is hyaline, septate. 3–4 μ thick and inter or intra cellular. At the point of zonations, the host cells become distorted, the fungal mycelium becomes ochraceous due to the accumulation of protoplasmic contents and aggregates to form stromatic masses in the intercellular spaces (Fig. I). From the stromatic mass, 3 or 4 hyphae, which are erect, often one-septate, 4–6 μ broad, come out of the stomata or by rupture of epidermis and divide again 3–4 times and form a hymenial layer, on which conidia are produced. The phialids measure 12–16 x 2–4 μ. Sometimes the fructifications are very close to each other. The conidia are produced acrogenously and are held together in a gelatinous mass. Conidia are elliptic or oblong, one celled, both ends rounded, some slightly broader in the middle with two oil globules, one at each end. They are light to olive green in colour with a deeper coloured wall and measure 7–8 x 2 μ. Enmass they look dark green.

(c) Pathogenicity: One and a half month old plants of cotton raised in glasshouse were inoculated with spore suspension and kept in the humid chamber. Numerous yellow coloured spots of pinhead size appeared on the leaves after 12–16 hours which soon increased in size to 5–6 mm. The colour of spots changed to tan. Fructifications were observed to appear after 36 hours on the undersurface and after 48 hours on the upper surface.

The plants were inoculated by spraying spore suspension, hyphal suspension, and by placing hyphal bits and sporodochia on the leaves already sprayed with sterile water and then covered with moist cotton wool. Five plants were kept as control i.e. only sprayed with sterile water. Infection obtained in each case is given below:

Spore suspension	...	20 per cent
Hyphal suspension	...	15 per cent
Spore masses	...	90 per cent
Hyphal masses	...	60 per cent
Control	...	0 per cent

The plant sprayed with spores and hyphal bits produced spots of yellow pinhead size, which soon enlarged while in the areas covered by sporodochia or hyphal masses, the entire portion became discoloured, infection spread easily and numerous fructifications made their appearance.

It was observed that during the crop season, a number of insects would visit and cause injury to the plants. Plants were therefore wounded with pin pricks and then sprayed with spore suspension. Cent per cent infection was obtained in case of wounded as against 20 per cent obtained in unwounded ones.

Dysdercus cingulatus (Red Cotton bug) was collected from the field, fed on healthy leaves for two days, later some of these were left on culture plates for 4 hours and then transferred to plants kept in humid chamber. The other lot which had not been fed on the pathogen, was released on controls. While the controls were all free, 80 per cent of the plants

on which insects from culture were released developed infection, showing thereby that they help in the dissemination of the disease.

Plants of different ages varying from 15 days to six months old were inoculated after injury. No difference was observed in the number or extent of spots formed. Leaves, twigs and fruits were inoculated but the infection developed on leaves only and very rarely on other parts.

When the plants were inoculated in November during winter, no infection took place, as the temperature was below 20°C, but during summer or rainy season infection took place readily at temperatures ranging from 25°C–38°C. It was observed during these tests that a high relative humidity was necessary for successful infection, development and sporulation of this fungus. When 24 hours after inoculation, the plants were kept out of the humidity chamber, the spots did not increase in size and when plants with fully developed spots were kept outside the humidity chamber, no fructifications were formed.

Spores of the fungus germinate readily in tap water, but temperature was found to have considerable effect. The data (based on average of 100–200 spores) recorded 18 hours after putting the spores for germination are given below:

Temp.	% age germination	Av. length of germ tube (μ)
6–7°C	Nil	Nil
13°C	84.1	6.75
21°C	92.3	19.6
28.5°C	95.5	16.0
31°C	92.0	14.75

It was observed that germination started at 28.5°C after 3½ hours, at 31°C after 4 hours, at 21°C after 5 hours and at 13°C after about 10 hours. At 6–7°C the spores did not germinate but after 36 hours when they were placed outside i.e. at a temperature of 23–25°C, they germinated freely.

(e) Cultural Studies: Fungus was grown on various natural and artificial media such as Oat meal, Potato Dextrose Agar 2 per cent, Richards' medium, Brown's medium and Czapek's medium. The fungus grew well on all the media but the growth of the aerial mycelium was more luxurient on natural media. Very little sporulation occurred on Czapek's medium. The fructifications formed in concentric rings (Fig. II). The fungus exhibited optimum growth at 25–30°C whereas best temperature for sporulation was 30–35°C and at pH range of 4.5–6.0.

(f) Host Range: In nature this fungus has been encountered at Delhi on the following hosts: *Calotropis gigantia* (Asclepiadaceae); *Citrullus vulgaris, Cucumis melo, Luffa acutangula, Lagenaria siceraria,*

(*Cucurbitaceae*); *Crotalaria juncea*, *Dolichos lablab*, *Cyamopsis tetragonoloba*. *Glycine max* (Leguminosae) *Amaranthus blitum* (Amarantaceae) *Abelmoschus esculentus*, *Gossypium* spp. (Malvaceae) *Corchorus capsularis*, *C. triloculoris* (Tiliaceae) *Vitis vinifera* (Vitaceae), *Ricinus communis* and *Euphorbia helioscopia* (Euphorbiaceae).

In order to determine the host range of the fungus, plants belonging to Compositae, Solanaceae, Malvaceae, Cucurbitaceae, Leguminosae, Graminae, and Chenopodiaceae were inoculated. All got infected, though degree of infection and symptom picture varied slightly in some cases. While in some, water soaked lesions were formed, on others purplish margin of the lesions were clearly visible.

Isolates of the fungus obtained from cotton, guar, jute and cucurbits were cross inoculated but they all produced the same type of lesions. These results are at variance from those of Brooks (1945) who reported host specificity of the two isolates from Potato and Antirrhinums.

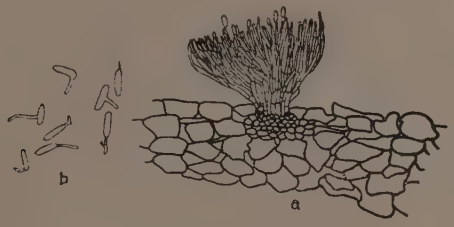

Fig. I. (a) T. s. through a fructification showing conidiophores & conidia.
(b) Germinating conidia.

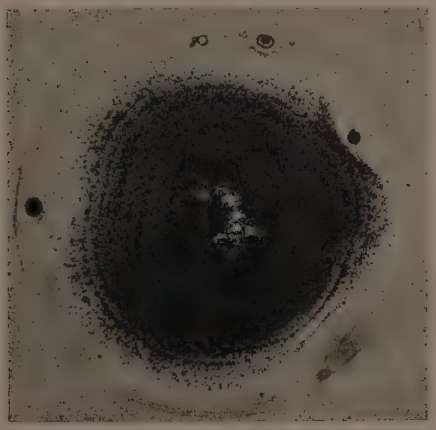

Fig. II. 20 days' old culture on P.D.A. showing zonate growth and sporulation of the fungus.

(g) Perennation of the Disease: The fungus has been found to overwinter on diseased leaves lying on the surface of the soil. The diseased leaves were placed in the open as well as buried in soil in the month of September and the viability of the fungus was tested from time to time. It was observed that the leaves buried in the soil rotted away in 2–3 months and the pathogen could not be isolated from them later, while those lying on the surface of the soil were found to have 40–50 per cent viable spores even after 8 months.

Control of the Disease: The toxicity of copper sulphate to the fungus was first studied in the laboratory. The spores were put for germination in 0.01 per cent, 0.05 per cent, 0.2 per cent, 0.2 per cent, 0.5 per cent and 1.0 per cent solutions. It was observed that except 0.01 per cent and 0.05 per cent all other doses were lethal for the spores. In 0.01 per cent solution, 90 per cent of spores germinated while in 0.05 per cent the spore germination was 40–50 per cent. In the field however, 1 per cent Bordeaux mixture was successful in controlling this disease.

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GROWTH PHASE METHOD FOR MUTANT-SCREENING IN COLLETOTRICHUM FALCATUM WENT

B. S. Bajaj and M. S. Chatrath

(Accepted for publication June 10, 1960)

Different techniques have been developed for irradiation of micro-organisms and isolation of mutants as the efficacy of a particular method depends upon the nature of the organism under study and the type of the mutants desired. Morphological mutants or the so called '*Visibles*', as the name indicates, are the easiest to detect whereas the physiological changes in the phenotype require special selection procedures. It is believed that in addition to the detectable phenotypic variability there may be a large number of inherited induced changes which are not observed either because their expression is low or suitable techniques for their detection are not available (Wagner & Mitchell, 1955).

Originally, Beadle and Tatum (1945) developed a method where mating of irradiated conidia of one strain with protoperithecia of another was made and single ascospores were picked up for the study of phenotypic variability, including deficiency on minimal medium. Later, Lein, *et al.* (1948) modified it by collecting the ascospores on solid minimal medium in petridishes and tested their deficiency again on the minimal medium. Markert (1952), working on *Glomerella*, made both plating and picking of irradiated conidia on complete medium. The cultures thus obtained were examined for morphological variation and further tested on the minimal medium for physiological deficiency. Other methods, e.g. Double plate technique (Lederberg and Tatum, 1946), Filtration enrichment method (Fries, 1947; Woodward *et al.* 1954; Catcheside, 1954) and Repetitive irradiation and mass culturing method (Clark & Webb, 1957; Sastry & Iyengar, 1959), are reported for different types of organisms. Boon *et al.* (1956) followed a method where small or retarded colonies obtained from the irradiated spores, were picked up with the aid of a stereoscopic microscope.

While working with *Colletotrichum falcatum* Went, the causal organism of red rot of Sugarcane, a method of irradiation and screening of mutants was developed and was found to be reasonably efficient. Carrier-free neutral solution of radioactive phosphorus was incorporated in 3 ml. oatmeal agar (40 g. quaker oats, 20 g. agar agar/litre) and sterilised at 15 lb. p.s.i. for 20 minutes. The level of initial activity of P^{32} ranged from 100 to 300 μc./ml. of the medium. A heavy conidial suspension of *C. falcatum* isolate No. 244 of the Indian Type culture collection from 15-day-old sporulating culture was prepared in distilled water and washed by centrifugation under aseptic condition. 0.1 ml. of this suspension was used for inoculating the slants having radioactive medium. The cultures were incubated at room temperature with proper shields. It was observed that the growth of this organism initiated after about 2 weeks

of inoculation when the initial activity of P^{32} was about 300 μc./ml. whereas in the controls the growth started after 2 days. However, in cultures with low level of activity, i.e., 100 μc./ml. there was no retardation in growth. The sporulation in the radioactive cultures was also markedly depressed. After about 30 days of incubation, spore suspension was prepared in distilled water from different parts of the slant, pooled together and repeatedly washed by centrifugation to reduce level of radiation as far as possible. The diluted spore suspension was mixed in warm oatmeal agar (used as complete medium) and distributed equally in sterilised petridishes in such a way that each plate received approximately 20–30 conidia so that the resultant colonies might not get mixed up. After two to three-day incubation at room temperature, colonies of different sizes appeared and a large number of transfers were made from these to slants of oatmeal agar and minimal medium (Dextrose 30 g. ; KNO_3 1.34 g., $MgSO_4$ 0.5 g.; M/30 phosphate buffer at pH 6.00) for study of morphological and physiological variability, respectively. For the minimal medium, high-purity grade chemicals and acid-cleaned glassware were used. Transfer of a colony with a small block of oatmeal agar directly to the slant of minimal medium has the advantage that even if the minimal medium does not support any growth, the variant would survive on the block of oatmeal agar and can, therefore, be subcultured further. Changes in colour, texture and nature of mycelium, colour, intensity and nature of spore masses and the microscopic characters, e.g., shape, size and other abnormalities in spores in comparison with unirradiated cultures were considered as criteria for morphological variation whereas restricted and slow growth on the minimal medium were taken as indication for physiological deficiency. Further analysis of deficiency was made using standard method (Beadle, 1947) by incorporating different growth factors into the minimal medium. The consistent behaviour of the variants in subsequent asexual generations was considered to be due to induced genetical changes.

As is clear from the table that within a limited number of transfers made, both for the study of morphological and physiological variability, a fairly good representation of variants of different types was observed. Unirradiated controls always yielded normal cultures. It is likely that even the mutants with low expression might get multiplied during the growth phase and thus become easily detectable. This method though not suitable for quantitative analysis, is useful for screening desirable mutants with specific characters, e.g., high antibiotic and other useful metabolite producing strains, mutants showing physiological deficiencies and those with specific morphological or pathological characters, as the expression of mutation is likely to get 'magnified'. Moreover, dividing nuclei in growth phase are considered more vulnerable to the mutagenic effect and, therefore, this method would appear also to help in enhancing mutation frequency. In subsequent experiments, it was observed that repeated irradiation of culture in growth phase with gamma rays from $cobalt^{60}$ source also helped in increasing mutation efficiency. The usefulness of this method is obvious from the fact that a mutant of *C. falcatum* with change in shape and size of spores detected in earlier experiments (Vasudeva *et al.*, 1958) was found again in 15 cultures out of the 150 dark mycelial types examined.

Table showing Population of Different Types of Variants of *C. falcatum* Induced by Irradiation with P^{32} in Growth phase.

Dose of P^{32}	Complete Medium							Minimal Medium				
	Total colonies picked up	Yellow pigmented	Dark mycelial type		Intermediate	Thin spore masses	Normal	Total colonies picked up	Growth completely restricted to OMA block	Slow growing	Dark mycelial types	Normal
			Normal Spores	Spores with change in shape and size								
100 μc./ml.	449	6	5	—	4	1	433	130	2	1	—	127
300 μc./ml.	424	1	135	15	111	2	160	135	3	32	57	43
272 μc./ml.	—	—	—	—	—	—	—	252	3	25	19	205

Summary

A method of irradiation of growing cultures of *C. falcatum* by incorporating P^{32} in the medium or with an external source of radiation and screening of morphological as well as biochemical mutants has been described. It is brought out that the method not only enhances mutation efficiency but also magnifies its expression and thus facilitates screening.

Acknowledgments: The authors wish to express their deep sense of gratitude to Dr. R. S. Vasudeva, Head of the Division, for suggesting the line of investigation, guidance, keen interest and helpful criticism throughout the course of this work. Thanks are also due to Dr. B. L. Chona, Mycologist for his valuable suggestions.

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LOSSES CAUSED BY THE BROWN LEAF-SPOT DISEASE OF RICE IN THE PUNJAB

KISHAN SINGH BEDI AND HARMOHINDAR SINGH GILL

(Accepted for publication February 10, 1960)

The Brown Leaf-Spot of rice, caused by *Helminthosporium oryzae* Breda de Haan, now occupies a prominent place in the Punjab among diseases, which affect this valuable food crop. Although this disease occurs every year, it particularly caused much damage to the rice crop during the year 1953 in the rice-growing areas of the State. As a result of the disease, it was not difficult to find fields, in which most of the leaves had become severely spotted. The heavy infection had also affected the panicles, and the result was that the grain formation was meagre. Some of the fields were observed to give from a distance a burnt or scorched appearance and the reduction in yield was reminiscent of the losses suffered by the crop during the year 1943 epiphytotic, which was considered to be one of the causes leading to the Bengal famine that year (Report of the Famine Inquiry Commission headed by Sir Woodhead, 1945).

Consequent upon the adoption of the 'Japanese Method of Rice Cultivation' on an extensive scale in the Punjab State in pursuance of the drive for the increased production of food-grains, the incidence of the disease is on the increase as a sequel to the addition of unusually heavy doses of artificial fertilizers and closer planting. This article deals with the losses estimated by the writers during the year 1954 at the Agricultural Station, Gurdaspur, which is situated in the heart of the main rice-growing areas of the Punjab.

1. REDUCED GERMINATION AS THE RESULT OF SOWING SPOTTED SEEDS: Six lots of 100 apparently healthy seeds with no visible spots on their husks, and a similar number of seeds with their husks severely spotted due to *Helminthosporium oryzae*, were taken in the case of 10 selected varieties and their germination was tested at the room temperature ranging from 27.5–31°C. The tests were carried out in Petri plates lined with moist filter paper. The data in respect of these tests are presented in table 1.

From table 1 it may be noticed that a substantial loss (11.0–37.3%) in germination is associated with the spotted condition of the seeds of different varieties. It may be pointed out that the spotted seeds, which did not germinate, were profusely covered with the mycelium and the spores of *Helminthosporium oryzae*, and a few of them, which succeeded in putting forth small radicles and plumules, were killed out-right within a few days of emergence. The rest of the seeds, that germinated, developed into seedlings bearing numerous lesions of the fungus. Padmanabhan *et al* (1948) have also reported that the infected seeds germinate poorly.

TABLE 1. Showing the percentage germination of apparently healthy and spotted grains of 10 different rice varieties

S. No.	Rice variety	Percentage germination of apparently healthy grains	Percentage germination of of spotted grains	Percentage loss in germination due to infection with *H. oryzae*
1.	349 Jhona	99.0	88.0	11.0
2.	370 Basmati	83.3	65.8	21.5
3.	246 Palman	99.0	81.8	17.2
4.	278 Sathra	94.8	82.6	12.2
5.	S. 20	96.8	84.8	12.0
6.	Begam Local	97.0	82.6	14.4
7.	Jhona Dhanoa	97.1	67.5	29.6
8.	360 Ranjha	94.5	77.0	17.5
9.	Toga 28	95.6	84.0	11.6
10.	China 62	94.1	56.8	37.3

2. LOSSES DURING THE SEEDLING STAGE OF THE PLANTS IN THE ABSENCE OF SECONDARY INFECTION. The assessment of loss due to the disease in the seedling stage was carried out in the case of the most commonly grown coarse variety of rice, namely, 349 Jhona. Six hundred apparently healthy seeds bearing no visible spots and a similar number of seeds artificially infested by smearing with the mycelium and the spores of the fungus, and a third similar lot of naturally infected seeds, were taken for this purpose. The tests were carried out in wooden flats. One hundred seeds were sown in each of the six flats employed for the above three categories of the seed. To preclude all chances of secondary infection, which would have otherwise vitiated the results, the flats were prevented from being exposed to the influence of rain by placing them under the roof of a 'verandah', whenever any shower was expected.

TABLE 2. Showing percentage germination and percentage seedling blight in the apparently healthy, the naturally infected and the artificially infested seeds in the case of variety 349 Jhona

Apparently healthy seeds	Average percentage germination	Percentage seedling mortality	Primary infections, as lesions on the surviving seedlings
Apparantly healthy seeds	99.0	0	0
Naturally infected seeds	94.5	9.1	100%
Artificially infested seeds	92.5	31.6	100%

An examination of the data presented in table 2 shows that the apparently healthy seeds give a very high percentage germination of 99

and remain free from any seedling mortality. This observation also hints at the successful multiplication of seed in a disease-free condition in dry areas, or in those of very low precipitation, such as Hissar and Rohtak, where the rice crop can be grown under irrigation to obviate the chances of primary infection from seed. In the case of naturally infected seed, there is a loss of about 5 per cent among the seedlings. Thus, under the conditions of the experiment, there has occurred a loss of about 15 per cent in the stand of the crop as the result of sowing diseased grains. In the case of artificially infested seed, there is not only a loss in germination to the extent of 7.5 per cent, but there is a very heavy mortality of 31.6 per cent among the seedlings, in addition. Ocfemia (1924) in Philippines found mortality in rice seedlings arising from rice grains affected with *Helminthosporium oryzae* to the extent of 10–58 per cent; while Tucker (1927) in varietal trials in Puerto Rico recorded deaths of seedlings from infected seed up to 15 per cent. Burnett (1949) reported that the leaves of rice seedlings were so heavily spotted that the nurseries presented a light-brown colour. Nisikado and Miyake (1922) state that in the seed-bed, the disease may attack about 90 per cent of the seedlings and sometimes all the plants are infected. It is significant to point out that in the writers' studies none of the surviving seedlings from among those arising from the artificially infested seed and the naturally infected seed were free from lesions, which varied from pin-head size to fully-developed spots. If the flats, in which such diseased seedlings were growing, had been exposed to rain, infection would have multiplied tremendously so as to scorch and kill all the seedlings.

3. Loss due to Infection at the Time of Panicle Emergence: Apart from the poor setting and the shrivelling of the seeds owing to the weakened state of the plants, the grains themselves are infected by the fungus. In severe cases, the glumes may become entirely covered with a dense black mass of its sporophores and spores. Such spikelets are generally sterile.

To determine quantitatively the loss in weight of the grains due to this disease, six lots of 100 seeds, each from apparently healthy plants, and a similar number of lots of seeds from heavily infected plants of 10 different rice varieties were taken separately and weighed for comparison. The data are presented in Table 3.

Table 3. Showing the weight of healthy and diseased grains of 10 different rice varieties

S. No.	Variety of paddy	*Weight of 100 healthy grains in grams	*Weight of 100 diseased grains in grams	Percentage loss in weight
1.	349 Jhona	2.40	2.29	4.58
2.	370 Basmati	2.17	1.87	13.8
3.	246 Palman	1.89	1.57	16.9
4.	278 Sathra	2.46	2.17	11.7
5.	S. 20	2.76	2.01	27.6
6.	Begam Local	2.40	2.05	14.5
7.	Jhona Dhanoa	2.34	1.97	15.8
8.	360 Ranjha	2.56	2.13	16.8
9.	Toga 28	2.72	2.38	12.5
10.	China 62	2.37	1.68	29.1

*Average of 6 repeats.

From the data set out in table 3, there may be noticed a loss in weight ranging from 4.58–29.1 per cent in the different rice varieties tested. Padmanabhan *et al* (1948) have recorded that the diseased condition of the grains due to *Helminthosporium oryzae* is associated with an appreciable loss in weight.

Summary

The nature of the damage caused by the disease is comparable with that caused by it in other countries like Japan, Philippines and Puerto Rico. The nature of the seedling blight has been observed to be similar to that recorded elsewhere. The diseased condition of the rice grains has been found to be associated with a loss in weight and in germination. The percentage loss in germination and in weight vary from 11.0–37.3 and 4.58–29.1, respectively.

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THE DAMPING-OFF ORGANISM, PELLICULARIA PRATICOLA (PAT.) FLENTJE, IN INDIA

H. K. Saksena

(Accepted for publication January 31, 1960)

Kotila (1929) first described *Corticium praticola* as distinct from *Corticium solani* (Prill. & Del.) Bourd. & Galz., the perfect stage of widely occurring sterile mycelium of *Rhizoctonia solani* Kuhn. The taxonomic position of *C. praticola* remained uncertain for a number of years because of its resemblance to *R. solani* in the imperfect stage. Flentje (1952) revived *C. praticola* by recording its occurrence in several areas in England and Australia. Later Flentje (1956) induced 28 isolates of *R. solani* from widely different localities to form the perfect stage and showed the complex species *R. solani* to be made up of two distinct species viz., *Pellicularia filamentosa* (Pat.) Rogers (*C. solani*) and *P. praticola* (Kotila) Flentje (*C. praticola*). *P. praticola* has been shown (Flentje and Saksena, 1957) to be unspecialized, attacking a wide range of hosts to cause severe rotting of hypocotyl and roots. This pathogen is now being recorded for the first time in India causing damping-off of seedlings of various host plants of economic importance.

Several fast growing isolates of *Rhizoctonia* were obtained in October, 1958, from damped off seedlings of *Antirrhinum, Linaria* and *Petunia* growing in the college botanical garden. The isolates were readily brought in pure culture on 2 per cent potato-dextrose-agar where they formed a dense, mealy greyish white surface growth. The mycelium is hyaline when young becoming light brown with age. The main running hyphae are 6.5–10 μ wide and the short side hyphal branches 8–12 μ. Small, yellowish brown round sclerotia up to 1.0 mm. in diameter develop within the medium and on glass sides and lid when the culture becomes old.

Hymenial development was induced by transferring about 25 day old potato-dextrose-agar cultures to Petri dishes containing 2 per cent corn meal agar. The inoculated plates were placed on flasks inverted in a glass trough containing water at the bottom. They were covered with bell jars and placed in diffused light near a window. The temperature ranged from 20–25°C. and the relative humidity was approximately 70–80 per cent inside the bell jars.

In about 17–25 days under these conditions, the isolates fruited on the surface of agar. The hymenium consists of small, white and flat plaques scattered over the plate with a tendency to form more freely near the margin. The plaques, made up of clusters of basidia, develop along the length of certain radiating hyphae only and on the lateral branches given out from the fertile hyphae. The simple and clavate basidia (Fig. 1) measure 13.5–21 x 5.8–7.9 μ. There are two to four, mostly three, tapering sterigmata per basidium. The sterigmata are 20.5–36 μ long

PENETRATION AND ESTABLISHMENT OF FUSARIUM OXYSPORUM F. PSIDII IN GUAVA ROOT[1]

J. C. Edward[2]

(Accepted for publication January 15, 1960)

The pernicious wilt disease of guava (*Psidium guajava* L.) in Uttar Pradesh, India, has been reported to be caused by *Fusarium oxysporum* Schlecht f. *psidii* Prasad, Mehta & Lal (1952). Based purely on symptoms of the disease the fungus has been considered a vascular wilt-causing pathogen (Das Gupta & Rai, 1947). The present paper gives a brief account of the work done on penetration of the root of guava seedlings by the fungus and its establishment inside the root tissue.

Seeds of "Safeda", a choice table variety of guava grown in Uttar

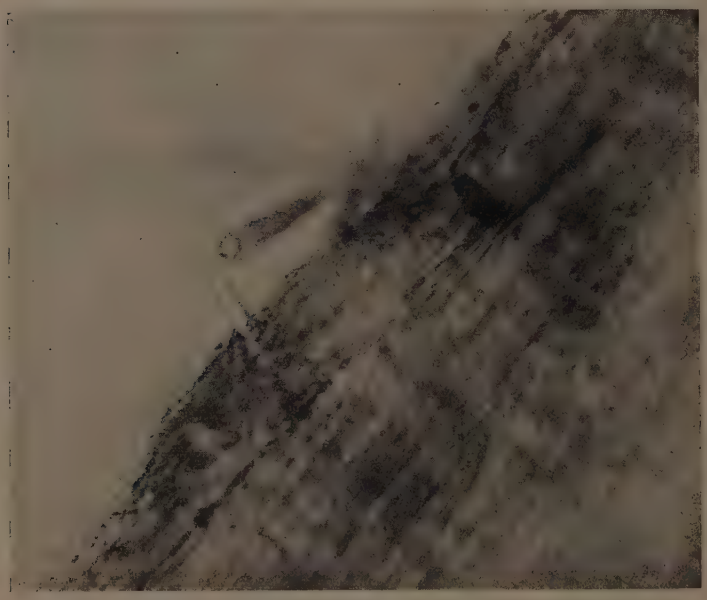

Fig. 1. Root penetration by germ tube of a single spore.

1 Paper No. 4217, Scientific Journal Series, Minnesota Agricultural Experimental Station.

2 Research Fellow, Department of Plant Pathology and Botany, University of Minnesota. This work was done while the author was on a Rockefeller Foudation Post-doctoral Fellowship.

Fig. 2. The arrows point to the regions of emergence of lateral roots where the germ tubes have penetrated.

Pradesh, India, were surface sterilized with 5 per cent sodium hypochlorite and germinated aseptically in Petri plates containing filter paper moistened with sterilized tap water. When the seedlings had reached the two-leaf stage, their roots were immersed in a spore suspension of *F. oxysporum* f. *psidii** in water and then transferred to test tubes containing nutrient agar medium. Three-week-old cultures of the fungus that had developed macroconidia were shaken with water and this was diluted until one ml. of the suspension had 6–7 million spores prior to immersion of the roots. The seedlings in the test tubes were incubated at room temperature (25–30 C) and their roots examined microscopically every day up to six days, after staining with carbol fuchsin (1%). The following observations were made. Root hairs were not penetrated by the fungus. In certain places direct penetration of the tap root by the germ tubes of individual spores was noticed without apparent damage to the cells penetrated or to those in their vicinity (Fig. 1). Spectacular *en mass* penetration of root by germ tubes of several spores was observed wherever secondary roots were on the verge of breaking through the piliferous layer of the taproot (Figs. 2 & 3). The vascular tissue of the infected young secondary roots was discoloured due to penetration of the hyphae. In certain cases discolouration extended even into the vascular tissue of the taproot.

* The culture of this fungus was kindly sent to me by Dr. R. S. Mathur, Government Plant Pathologist, Kanpur, Uttar Pradesh, India.

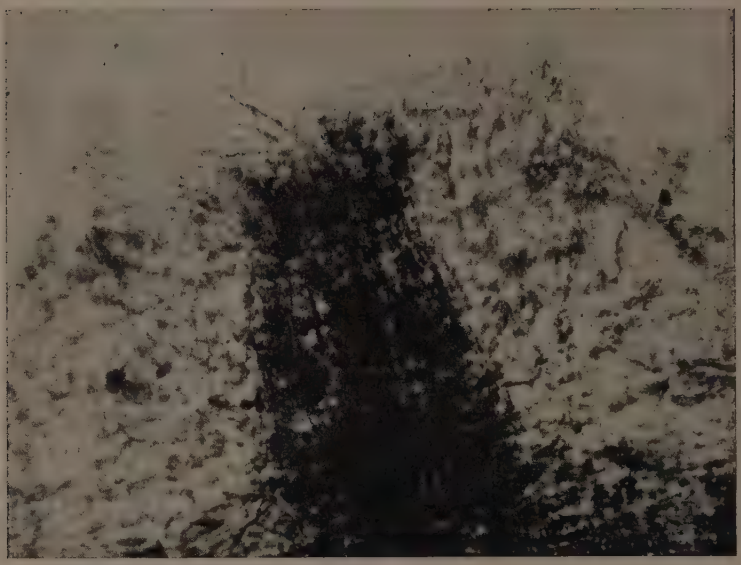

Fig. 3. *En mass* penetration of an emerging lateral root by several germ tubes. (Enlargement of infection focus 1 from Fig. 2.)

Microtome sections of the roots of wilted plants which had been growing in artificially infested soil under green house conditions showed the presence of fungus hyphae in the vascular tissue, absence of starch granules in parenchymatous cells of the stele and their discolouration, and plugging of some xylem vessels by dark brown gum (Fig. 4).

Root hair penetration by the fungus was not observed although it has been reported by Tisdale working with flax-wilt fungus (1917). Direct penetration of the fungus through the piliferous layer as noted in this investigation has been recorded by Dharmarajulu (1932) working with cotton wilt pathogen. The penetration of hyphae *en mass* through punctures in the piliferous layer caused by lateral root outgrowth, should it occur in nature, is doubtless a quicker and more effective means of entry into the vascular tissue. Hitherto the pathogen was considered a vascular wilt-causing fungus with only indirect evidence (Das Gupta & Rai, 1947). However, as observed in the present study, the occurrence of fungus hyphae in the vascular tissue accompanied by plugging of some xylem vessels by dark brown gum confirmed the vascular nature of the fungus.

Summary

Fusarium oxysporum f. *psidii* Prasad, Mehta, and Lal penetrates directly through the piliferous layer of roots of guava seedlings or through openings in the piliferous layer caused by growth of young secondary roots.

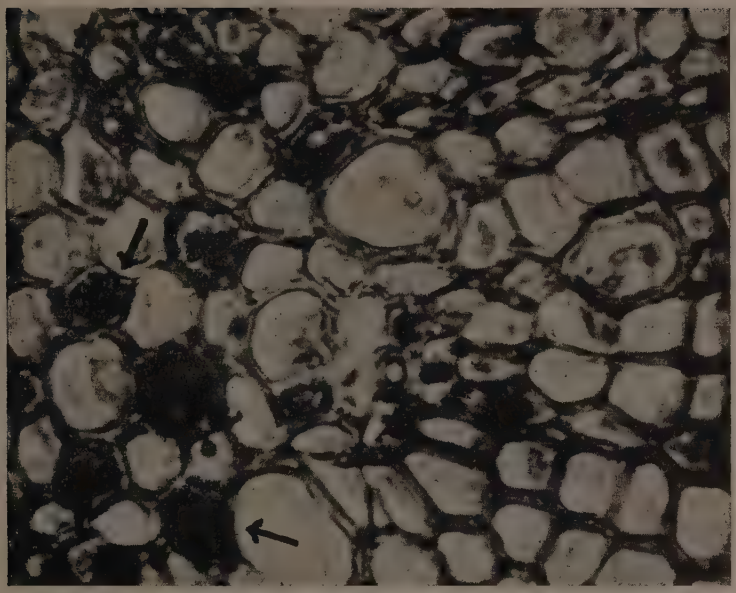

Fig. 4. Cross section of infected plant-root showing vascular hyphae and plugging of xylem vessels by gum. The arrows point to the plugged vessels.

Hyphae of the fungus were found in the xylem vessels of roots of artificially infected plants.

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Phytopathological Notes

Lettuce Mosaic. by T. K. Nariani and P. S. Pathanian. A mosaic disease of lettuce (*Lactuca sativa* L.) has been found to be of common occurrence at the Indian Agricultural Research Institute for the last few years. The disease is characterised by mosaic mottling of the leaves accompanied by scorching of the leaf margins and defective hearting in advanced stages of crop. The whole plant as a rule appears a little paler in colour. Irregular chlorotic and dark green areas are interspersed all over the leaf surface (Fig. 1). Very often the leaves show slight puckering. As the leaves grow old the mosaic symptoms become less conspicuous although the younger leaves continue to show the mosaic symptoms. With the onset of the hot season during the months of March-April there is a tendency for the symptoms to be masked.

Lettuce Mosaic

The disease was successfully transmitted by sap inoculation to healthy lettuce plants under glass house conditions using carborundum powder as an abrasive. The percentage of infections could, however, be increased by expressing the juice in 0.5 per cent sodium sulphite solution. The virus was readily transmitted by *Aphis gossypii* Glov., *A. evonymi* Fabr. and *Myzus persicae* Sulz. as also through the seed obtained from diseased plants, the percentage of seed transmission being about 2.

In the host range studies, out of several plant species belonging to 9 different families that were tested, the virus was transmitted only to *Pisum sativum* which produced transient mottle and *Lathyrus odoratus* and *Gomphrena globosa* which proved to be symptomless carriers.

The virus has a thermal-death-point of 50–55 °C, a dilution-end-point between 1 : 50 and 1 : 100 and longevity *in vitro* of 24–48 hours at room temperature (23–24 °C).

The causal virus differs from the yellow mosaic virus reported earlier on lettuce (Vasudeva *et al*, 1947) but resembles the common lettuce mosaic virus (*Marmor lactucae* Holmes or *Lactuca* virus 1 Smith) reported from U.S.A. (Grogan and Bardin, 1950), Great Britain (Ainsworth and Ogilvie, 1939) and New Zealand (Chamberlain, 1948; Fry, 1952).

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest and helpful suggestions during these investigations.

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Nematode Root Injury of Sugarcane: —B. L. Chona and Gopal Swarup. A serious disease of sugarcane was observed in Nellikuppam area (S. Arcot, Madras) during the 1958–1959 season. The canes were drying in a peculiar fashion, without exhibiting the typical Wilt symptoms. Such canes were found to be hollow from inside, particularly the top portion. Healthy as well as the diseased canes were present in the same clump. The root system in the diseased canes was not well developed and showed typical stubby roots accompanied by root rot injury. In two varieties, Co. 1,001 and H32/8,560, the roots were so badly damaged that the plants could be easily pulled out. Lesions were found to be present on young new roots also.

Diseased canes and soil samples from around the rhizosphere of such canes were collected in January, 1959 for examination. The analysis of the root and soil samples showed the presence of a large population of a parasitic type of nematode which has been identified to belong to the genus *Tylenchorhynchus*. Besides this, nematodes of two other genera viz. *Hoplolaimus* and *Pratylenchus* were also found during the analysis of these samples but their population was very low in comparison to *Tylenchorhynchus* sp. In one of the cultures maintained in the glasshouse, however, root-knot symptoms were observed on a few roots of the cane variety H32/8,560. In view of the presence of parasitic nematodes around the rhizosphere of diseased canes, preliminary observations were made on the

initial nemic population as well as the population level of the nematodes in the same cultures after a period of two months.

In the first instance soil samples, collected from the rhizosphere of the three diseased sugarcane varieties, namely Co. 1,001, Co. 449 and H32/8,560, were analysed according to the modified Baermann funnel technique (Christie and Perry, 1951) and population counts of the nematodes isolated were taken. After this initial population count, the remaining soil was mixed with local soil (approximately 50: 50 proportion) and filled in 10 inches pots and planted with healthy setts of Co. 449, Co. 1,001 and H32/8,560. After about 2 months, soil samples were drawn out from these pots and the level of population of various types of nematodes was determined. Three soil samples were taken from each pot and the nema population determined. The average of the counts of nematode population, both initial as well as after 2 months' period, are given in the table.

TABLE. Nematode population* of soil samples collected from the rhizosphere of 3 sugarcane varieties

Name of sample	Total No. of nematodes		Total No. of saprophytes		Total No. of *Hoplolaimus* sp.		Total No. of *Pratylenchus* sp.		Total No. of *Tylenchorhynchus* sp.	
	Initial	After 2 months	Initial	After 2 months	Initial	After 2 months	Initial	After 2 months	Initial	After 2 months
Co, 1,001	631	1.028	564	928	1	4	...	...	66	96
Co. 449 ...	586	835	443	634	23	26	31	38	89	137
H 32/8,560 ...	1,009	1,800	33	41	4	5	7	4	965	1,750

* The population given is for 200 g. of soil.

It is evident from the data that, amongst the parasitic nematodes, *Tylenchorhynchus* sp. formed the major population and that none of the three cane varieties appear to be a congenial host for *Hoplolaimus* sp. or *Pratylenchus* sp. as they were not able to multiply on these varieties. It would thus appear that *Hoplolaimus* sp. or *Pratylenchus* sp. are not responsible for the diseased condition in sugarcane observed at Nellikuppam. *Tylenchorhynchus* sp. however, did multiply on all the 3 varieties of sugarcane, though to a very much less extent on Co. 449 and Co. 1,001 as compared to that on H32/8,560, which accounted for approximately 97 per cent of the total nemic population at the end of the 2 months' period. Of the other two cane varieties, Co. 449 appears to be a slightly better host for *Tylenchorhynchus* sp. than Co. 1,001.

A somewhat similar disease of sugarcane was reported from Louisiana (U.S.A.) by Birchfield (1953) wherein *Tylenchorhynchus martini* Fielding was found to be associated with the roots of the diseased canes.

Pathogenicity of the nematode on sugarcane was also established by Birchfield and Martin (1956). The fact that the disease was observed in the fields at Nellikuppam during the rainy season indicates that lack of soil moisture is not the cause of the drying up and hollowing of the canes. It is possible that the root lesions observed on the diseased canes may be due to the nematode *Tylenchorhynchus* sp. The presence of large numbers of this nematode around the rhizosphere of diseased canes further indicates this possibility. At the present moment, pathogenicity tests, with this nematode on sugarcane varieties, are in progress and at the end of these experiments it will be possible to throw more light on the role of nematode as such, or in association with other root-rotting fungi, in causing the diseased condition in the sugarcane crop observed at Nellikuppam during 1958–59.

Acknowledgements: The authors wish to record their grateful thanks to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, for his keen interest, helpful criticism and encouragement and Dr. R. D. Rege, Agricultural Adviser, Parry and Co., and the management of the Nellikuppam Sugar Factory for kindly providing the necessary facilities and the required seed cane material and soil samples for study.

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Inhibition of Radish Mosaic Virus in Brassica Spp. by Ultraviolet Irradiation—S. P. Raychaudhuri and H. C. Prasad. Radish mosaic virus (Raychaudhuri and Pathanian, 1955) produces local lesions on several *Brassica* spp. It is a stable virus and was, therefore, considered suitable for studying the inactivation by ultraviolet irradiation. The data regarding the observations made thereon are reported in this note.

Material and Methods: Healthy and inoculated test plants of *Brassica juncea*, *B. campestris* var. *toria* and *B. campestris* var. *sarson* were exposed to ultraviolet light and inhibition was studied by local lesion counts. Extracts of radish mosaic virus were obtained by crushing the young diseased leaves of radish plants grown in the glass house. The juice was then centrifuged at 3,500 r.p.m. for 30 minutes in a Wifug type centrifuge, which yielded a clear light golden coloured liquid after

removal of the extraneous matter. This was then diluted to 1 : 10 which was used in all experiments. A glass spatula specially prepared for the purpose of inoculation was dipped only once in the diluted virus extract and gently passed over the leaf of healthy plant by one gentle and uniform stroke. Local lesions appeared after 8 to 15 days when counts were taken. The maximum temperature in the glass house varied from 37° to 43°C. and the minimum temperature varied from 16° to 26°C.

For exposure to ultraviolet light, an Alpine Sun Lamp, Model IX* running on 220 volts DC in which dosage could be calculated in the minimum units** was used. When the material was exposed for a period of 45 seconds at a distance of 24 inches from the arc tube, it received a minimum unit dose.

Experimental : Leaves of the test plants were inoculated before and after exposure to ultraviolet light for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes, always placing the inoculated leaves approximately at a distance of 24 inches from the arc lamp. In this preliminary test it was noticed that there was marked reduction in the local lesion counts when exposed for 15 and 30 minutes in comparision with the control which was not exposed. Exposure for 35 minutes or more had adverse effect on the plant itself while exposure for a period of 10 minutes or less did not appreciably reduce the local lesions. The plants were, therefore, exposed to ultraviolet light for 15 and 30 minutes (20 and 40 m.u.d.) (a) before inoculation, (b) after inoculation and (c) one week after inoculation. Unexposed plants were used as control.

The inhibition, expressed as percentage reduction in lesion counts, was calculated in all experiments by dividing the number of lesions produced by the control, multiplying the quotient by 100, and substracting the product from 100. In all experiments the extracts of the virus were adjusted to pH 7 by addition of sufficient 1.0 M phosphate buffer. The data are shown in the table.

The data* presented in the table indicate that the number of local lesions on the three hosts i e *Brassica juncea*, *B. campestris* var. *toria* and *B. campestris* var. *sarson*, which were exposed to 20.00 and 40.00 m.u.d. before and after inoculation was appreciably reduced. Marked inhibition was noticed when exposed to 20.00 m.u.d., the reduction percentage being 96.95, 96.10 and 96.60, respectively when exposed immediately after inoculation. However, inhibition only to extent of 9.8 per cent, 16.15 per cent 16.6 per cent was observed when exposed before inoculation. Also, exposure one week after inoculation indicated appreciable reduction in local lesion counts, that is, 58.15 per cent, 57.85 per cent and 53.70 per cent, respectively. When the same three hosts were exposed to ultraviolet irradiation at 40.00 m.u.d. after inoculation, marked reduction was observed in the percentages of local lesion counts the inhibition being 97.4 per cent, 96.75 per cent and 96.7 per cent, respectively.

* The ultraviolet lamp was used in the Experimental Physics Laboratory, I. A. R. I.

** A minimum unit of dosage of exposure to ultraviolet irradiation is 5,22,000 ergs per sq. c. m. of the Erythema producing ultraviolet rays.

TABLE. Effect of Irradiation of Intact leaves of *Brassica* spp., on the local lesions produced by Radish Mosaic virus

Exp. No.	Time of exposure	Treatment	*Brassica juncea*		*Brassica campestris* variety *toria*		*Brassica campestris* variety *sarson*	
			Local lesions on 9 leaves	Per cent reduction	Local lesions on 12 leaves	Per cent reduction	Local lesions on 12 leaves	Per cent reduction
I.	15 minutes or 20.00 m.u.d.	Exposure before inoculation	457	8.7	565	13.5	485	17.3
		Exposure after inoculation	15	97.1	23	96.5	15	97.5
		Exposure after 1 week	227	55.30	295	54.9	263	55.2
		Control (not exposed)	507	—	653	—	586	—
	30 minutes or 40.00 m.u.d.	Exposure before inoculation	387	18.6	586	13.2	570	6.1
		Exposure after inoculation	13	97.30	19	97.2	17	97.2
		Exposure after 1 week	157	67.0	258	61.5	245	59.7
		Control (not exposed)	475	—	675	—	607	—
II.	15 minutes or 20.00 m.u.d.	Exposure before inoculation	468	10.9	508	18.8	505	15.9
		Exposure after inoculation	17	96.8	27	95.7	26	95.7
		Exposure after 1 week	205	61.0	147	60.8	87	52.2
		Control (not exposed)	525	—	625	—	600	—
	30 minutes or 40.00 m.u.d.	Exposure before inoculation	425	15.2	545	11.4	482	18.5
		Exposure after inoculation	13	97.5	23	96.3	23	96.2
		Exposure after 1 week	227	54.7	298	51.6	247	58.3
		Control (not exposed)	501	—	615	—	591	—

When the plants were exposed before inoculation the percentage reduction in local lesion count was 16.9, 12.35 and 12.3, respectively. Also reduction of local lesion counts to the extent of 60.85 per cent, 56.55 per cent 59.00 per cent. respectively was noticed when exposed one week after inoculation.

CONCLUSIONS: When the leaves of *Brassica juncea*, *B. campestris* var. *toria* and *B. campestris* var. *sarson*, inoculated with radish mosaic virus were exposed to ultraviolet light for 15 and 30 minutes (20.00 and 40.00 m.u.d.) the virus was almost completely inactivated, while exposure after 7 days indicated appreciable inhibition. However, exposure before inoculation did not inhibit the viral penetration and multiplication, proving thereby that irradiation of the host cell does not change the host susceptibility but inactivates the virus to the limit to which the ultraviolet light could penetrate.

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A Preliminary study of Crown Rust in India.—Gopal Swarup, M. M. Payak and D. P. Misra. *Puccinia coronata* Corda on cultivated Oats (*Avena sativa*) was first recorded in India by Butler in 1907 at Pusa, where it was found to cause a minor epidemic (Butler and Bisby, 1931). Subsequently P. C. Kar recorded it from Rangpur in Bengal in 1922 (Padwick and Khan, 1944) and Roy (1948) from Berhampore. Since then there have been no reports of its occurrence until in 1956 when a collection of rust on Oats was obtained from Kalimpong (West Bengal). This collection was studied for its morphology and pathogenicity. The uredia are amphigenous, elongate, separate or coalescent, pulverulent, and orange in colour. Urediospores are globose, ovate-elliptic, with hyaline and finely echinulate wall. They are orange in colour with obscure germ pores and measure 18–27 x 15–18 μ. Oat varieties—Minrus, Joanette, Richland, and Victory—have been found susceptible while it fails to infect local Lyallpur Oats. This rust collection is also able to infect the common wild species of Oats—*Avena fatua*.

Padwick and Khan (1944) have stated that two types of acciospores occur in aecia found on *Rhamnus* in India which are externally similar.

In one collection on *R. virgata* they measured 15.7–20.5 x 19.3–25.3 μ and in another from *R. dahurica* their dimensions were: 12.0—14.5 x 14.5–16.9 μ. On *Rhamnus* round about Simla also two types of aeciospores have been found. The respective sizes are: 21–27 x 15–19.5 μ and 15–18 x 12–16.5 μ. Uredial and telial collections of *P. coronata* from Simla and adjacent locations have been made on *Brachypodium sylvaticum*, *Helictotrichon virens*, and *Agrostis* sp. The telia on *B. sylvaticum* are naked and epiphyllous, while on the other two grasses they are covered as is the case in the true Crown Rust of Oats.

Teliospores of the rust found on *B. sylvaticum* were collected after the thawing of the snow (January, 1960). They were germinated and inoculated on *Rhamnus* plants. Pycnia developed readily within 8–10 days. Pycnial exudate was intermixed aseptically and aecia developed in due course mostly on the apical portions of young shoots and occasionally on leaves also. The aeciospores measured 15–18 x 12–15.5 μ, thus falling within the category of small-sized aeciospores. It was thus established that the small-sized aeciospores observed by previous workers on *Rhamnus* belong to the rust found on *B. sylvaticum*. In nature also aecia on *Rhamnus* containing such small-sized spores have been found associated with telial infections on the leaves of *B. sylvaticum*. Because of its distinctive morphological characters, the rust on this grass deserves to be ranked as a separate variety or *forma speciales* of *P. coronata*. Barclay (1891) had already named it as *P. coronata* var. *himalensis* Barcl., which name, in the light of present work, should be considered as valid for this rust. Preliminary work has shown that in pathogenicity also the rust appears to be restricted to *B. sylvaticum* only.

Aecia on *Rhamnus* containing big-sized aeciospores were collected from Prospect Hill, Simla. These were inoculated on four varieties of Oats (Victory, Joanette, Minrus and Richland) as well as on local Lyallpur Oats. On the first four varieties, moderate uredial infection was obtained after 10 days of inoculation. However, local Oats failed to get infected. The pathogenicity of these *Rhamnus* aecia thus appears to be similar to that of the Oat rust collected from Kalimpong.

Further work to determine whether these aecial collections containing big-sized aeciospores on *Rhamnus* and uredial and telial collections on *Agrostis* and *Helictotrichon* and the uredial collection from Kalimpong bear any relationship to, or are identical with, the true Crown Rust of Oats, is in progress. These studies are also expected to throw light on the role of *Rhamnus* in causing rust infection on cultivated Oats in India.

ACKNOWLEDGEMENTS: We wish to record our grateful thanks to Dr. R. S. Vasudeva for suggesting the problem and for his guidance and helpful suggestions during the course of these investigations.

Our grateful thanks are due to Mr. V. C. Lele also for his keen interest throughout these studies.

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A Bacterial Leaf-spot Disease of Teramnus Labialis.—V. V. Bhatt, V. H. Pawar, and R. S. Sukapure. In the course of the studies on phytopathogens in Poona, a leaf spot disease of bacterial origin was observed in *Teramnus labialis* Spreng. The disease first appears as hypophyllous minute water-soaked spots. After about 5–6 days, the infection becomes visible on the upper surface also as round to angular pale-brown spots which are surrounded by yellow halo. The yellow zone of the upper surface corresponds to the water soaked areas on the lower surface. The necrotic spots which initially measure about 0.5 to 1.0 mm. increase to about 2.0 to 3.0 mm. and become dark brown to black. When numerous spots coalesce, infected leaves become pale yellow and get defoliated.

The pathogen was isolated by usual poured plate method using potato dextrose agar. Small, shining, yellowish colonies appear after 48 hours incubation at 28°C. Single colonies when subcultured and tested, proved pathogenic to the suscept on artificial inoculation.

Host Range: The pathogen infects only *T. labialis* and not *Alysicarpus rugosus* DC., *A. vaginalis* DC., *Arachis hypogaea* L., *Cassia tora* L., *Cajanus cajan* Millisp., *Cicer arietinum* L., *Crotolaria juncea* L., *Cyamopsis tetragonoloba* (L) Taub., *Desmodium diffusium* DC., *Dolichos biflorus* L., *D. Lablab* L., *Lathyrus odoratus* L., *Medicago sativa* L., *Phaseolus bilobata* L., *P. mungo* var. *radiatus* L., *P. trilobus* Ait., *P. vulgaris* L., *Pisum sativum* L., *Sesbania aegyptica* Prain., *Soja max* (L) Piper., *Stizolobium deerengianum* Bort., *Tamarindus indica* L., *Tephrosia purpurea* Pers., *Trifolium foenum-graecum* L., *Vigna catjang* Walp., etc.

Nomenclature: From the morphological, cultural and biochemical characters it is evident that the pathogen under study is a *Xanthomonas* species, and distinct from the other *Xanthomonas* species on Leguminosae. The species under study is host specific and is presented as a new species with a name *Xanthomonas teramnii.*

Technical Description: Short rods, measuring 0.7 x 1.8 μ; single polar flagellum; capsulated; non spore former; Gram negative; potato dextrose agar colonies circular with entire margin, smooth, butyrous, raised and Baryta yellow (R), measuring about 20 mm. in diameter after

8 days at 28 °C; good growth without pellicle in nutrient broth, turning the latter brown after 36 to 48 hours at 24–28 °C., on nutrient agar with 2 per cent glucose, the growth is excellent butyrous and sulphur yellow (R) turning the medium brown after 36–48 hours at 24–28 °C; gelatin slowly liquified; starch strongly hydrolysed; casein digested, hydrogen sulphide and ammonia produced from peptone; indol not produced; milk peptonised and litmus slightly reduced; good growth in synthetic nitrate and Czapek's media; nitrite and ammonia not traceable from nitrate; good growth and acid without gas from dextrose, dextrine, galactose, glucose, lactose, levulose, maltose, sucrose, starch, xylose; fair growth and slight acid from glycerine and mannitol; good growth with alkaline reaction in sodium acetate, sodium citrate and sodium lactate; no growth in dulcitol, salicin and sorbitol; sensitive to penicillin at and above 2,000 u/ml. giving about 15–17 mm. zone of inhibition on assay agar medium; thermal death point near about 50 C.

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Suspected plant parasitic nematodes in certain sugarcane soils of Madras State—Kishan Singh. In January 1960, 39 soil and sugarcane root samples were collected from certain sugarcane fields in Madras State from where reports were received of driage of leaf tops and pithiness of canes. Nematodes present therein were isolated with the modified Baermann funnel technique and by dissecting the roots (Christie, 1951, Dieter, 1954, Taylor *et al.* 1955). Among those isolated and identified as the likely plant parasites *Hoplolaimus* n. sp., *Pratylenchus pratensis*, *Meloidogyne javanica*, *Tylenchorhynchus* sp., *Criconemoides* sp., *Helicotylenchus nannus* and *Helicotylenchus erythriane* and *Xiphinema citri* formed the majority.

These nematodes were observed in and around the roots of Co. 449, Co. 658, Co. 956, Co. 1,146, and Pindar in Edyanvelly, Co. 419 in Cuddalore and Kombupalayam and Co. 658 and Co. 1,001 in Kongarayanur. At Edyanvelly, the occurrence of nematodes was comparatively much less around the root zone of Co. 956 than of other varieties. *Phyllanthus niruri*, a weed commonly present in sugarcane fields in Edyanvelly, was observed to be parasitised by *Meloidogyne* sp.

Species of *Fusarium*, *Rhizoctonia* and *Pythium* were isolated from several such roots as were apparently infested with nematodes. This indicates the possibility of the existence of fungus-nematode associations, forming disease complexes (Srinivasan, 1958). This aspect deserves further investigations.

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Sugarcane Breeding Institute, Coimbatore for their help in collection of the soil and root samples.

Indian Institute of Sugarcane Research,
Lucknow.

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A new physiologic race of Puccinia graminis tritici (Pers.) Erikss. and Henn. in India. Sheodhan Singh, V. C. Lele and R. S. Vasudeva. A new physiologic race of black rust of wheat, *Puccinia graminis tritici* not hitherto recorded from India has been picked up from rust collections of 1958-59 crop received from several states of Peninsular India. The first indication of new race was obtained while analysing a rust collection from Gwalior, Madhya Pradesh on a wheat variety, Pissi local and from rust collections from Dharwar, Mysore. The types of infection produced by the new race on the differential varieties are given below:—

Place of Collection	Original Host	DIFFERENTIAL VARIETIES											
		L. Club	Marquis	Reliance	Kota	Arnautaka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
Gwalior	Pissi Local	4	0;-2	0;	0;	4	4	4	4	4	3-4	0;-1	0;-1

This race differs from race 24 by the resistant type of infection produced on Marquis. It resembles race 14 of the International Register of black rust races.

The geographical distribution of this race was found to be in Madhya Pradesh, Rajasthan, Bombay State, Mysore State and Andhra Pradesh—thus, so far restricted to Peninsular India.

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Race 17 of brown rust of wheat: A new record for India—L. M. Joshi, Gopal Swarup, K. R. Sreekantiah and R. S. Vasudeva. A new race, resembling race 17 of *Puccinia triticina* Eriks. of International key has been isolated from various collections of rust, from the crop year 1956–57 from India. In the very first year of its appearance in the country, the race was recorded from the states of Uttar Pradesh, Bihar, Madhya Pradesh, Bombay, Madras and Andhra. Its frequency was 12.7 per cent during the year.

Reactions of one of the isolations found in India are compared with race 17 of the International key in the following table:

Locality and Original host	Stock Collection or Insolation	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat
Wellington Wheat var.?	Brevit (1–2)	4	0;–2	0;–2	0;–1	0;–2	0;–1	4	0;–1
——	Race 17	4	0	0	0	0	0	4	0

Race 17 has been picked up from most of the states of the country during subsequent years.

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Race 162 of brown rust of wheat: A new record for India.—D. P. Misra, V. C. Lele and R. S. Vasudeva. During the analysis of brown rust samples collected from crop year 1955-56, a sample from Gwalior (Madhya Pradesh) on wheat variety N.P. 720, yielded race 162. Reactions of the eight differential varieties to the isolate from India and to the race 162 of the International key are set out in the following table for comparision:

Locality and Original host.	Stock collection or Isolation	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat
Gwalior (M.P.) N.P. 720	Mal. (2)	0;–2	4	4	3	4	4	X	4
——	Race162	I	3	4	4	4	4	X	4

Race 162 is different from race 77 for its resistant type of reaction on Malakof. Reactions on other seven differential varieties are more or less identical with these two races. All other races previously known in India i.e. 10, 11, 20, 26, 63, 70, 106, 107, & 108 are uniform for the resistant type of reactions produced on Mediterranean and Democrat which varieties are susceptible to races 77 and 162.

Race 162 has shown increasing prevalence in subsequent crop-years.

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